

Bulletin No. 41 from the Department of Surgery,

University of Lund, S-221 85 LUND

1983

HYPERTHERMIA AND LIVER TUMORS

An experimental study in rats



Anders Hugander

HYPERTHERMIA AND LIVER TUMORS

An experimental study in rats

Anders Hugander

leg läk, Smld

Akademisk avhandling

som för avläggande av doktorsexamen i medicinsk vetenskap

vid Medicinska Fakulteten vid Universitetet i Lund

kommer att offentligens försvaras å Föreläsningssal 1,

Centralblocket, Lasarettet i Lund,

fredagen den 30 september, kl 09.15.

Organization LUND UNIVERSITY Department of Surgery University of Lund S-221 85 LUND		Document name DOCTORAL DISSERTATION	
		Date of issue 1983-09-30	
		CODEN: LUMEDW/(MESL-1022)/1-130/1983	
Author(s) Anders Hugander		Sponsoring organization	
Title and subtitle HYPERTHERMIA AND LIVER TUMORS. An experimental study in rats.			
Abstract The present study introduces a technique for induction of general and local hyperthermia in rats. The study was performed on normal Wistar rats and rats with inoculated liver tumors (N-methyl-N-nitrosoguanidine-induced adenocarcinoma). Hyperthermia was induced by microwave irradiation at 2 450 MHz. Disturbance of the continuous temperature registration was avoided by alternating irradiation with temperature registrations. A microprocessor based feed-back system controlled the hyperthermia treatment. Total body hyperthermia at temperatures above 41.5 °C caused an increasing mortality. Evidence of liver cell damage and increased lysosomal activity indicated a specific sensitivity of the liver to higher temperatures. No tumoricidal effect of total body hyperthermia could be demonstrated in liver tumors. After local hyperthermia treatment at 42 °C a tumoricidal effect was demonstrated by tumor volume measurements and histopathological examination. The addition of an intravenous infusion of 5-fluorouracil (20 mg x kg⁻¹) did not further increase the tumoricidal effect of local hyperthermia. An increased mortality and a fast decline in the serum concentration curve of 5-FU after combined treatment indicated that drug metabolism was affected by heat. In order to study blood flow in the liver during local hyperthermia the 133-Xenon clearance method was adopted for studies in rats. The elimination rate (k₁) of the fast component of the excretion curve was supposed to represent the relative liver blood flow. An overall decrease in liver blood flow was observed during local hyperthermia (42 °C). Tumor blood flow did not return to prehyperthermic values after heating, suggesting at least a temporary disturbance in the tumor circulation.			
Key words Microwaves; general hyperthermia; local hyperthermia; liver tumors; 5-fluorouracil; liver blood flow; Xenon.			
Classification system and/or index terms (if any)			
Supplementary bibliographical information		Language English	
ISSN and key title		ISBN	
Recipient's notes		Number of pages 130	Price
		Security classification	

Distribution by (name and address)
 Anders Hugander, M.D., Department of Surgery, University of Lund, S-221 85 LUND, Sweden

I, the undersigned, being the copyright owner of the abstract of the above-mentioned dissertation, hereby grant to all reference sources permission to publish and disseminate the abstract of the above-mentioned dissertation.

Signature

Anders Hugander

Date

1983-08-29

From the Department of Surgery, University of Lund,

S-221 85 Lund, Sweden

HYPERTHERMIA AND LIVER TUMORS -

AN EXPERIMENTAL STUDY IN RATS

by

Anders Hugander

Lund 1983

To my family



This thesis is based on the following papers:

- I. EXPERIMENTAL SET-UP FOR STUDIES OF MICROWAVE-INDUCED HYPERTHERMIA IN RATS.
M Bolmsjö, Lo Hafström, A Hugander, P-E Jönsson and B Persson.
Phys Med Biol 27(3):397-406, 1982.
- II. TOTAL BODY HYPERTHERMIA INDUCED BY A COMPUTERIZED MICROWAVE TECHNIQUE: STUDIES IN NORMAL RATS AND IN RATS WITH LIVER TUMORS.
A Hugander, G Carlsson, Lo Hafström, P-E Jönsson, B Hultberg, M Bolmsjö and B Persson.
Anticancer Res 3:161-166, 1983.
- III. LOCAL HYPERTHERMIA IN TREATMENT OF EXPERIMENTAL LIVER TUMORS.
A Hugander, Lo Hafström, P-E Jönsson, M Bolmsjö, B Persson and U Stenram.
Cancer, in press, 1983.
- IV. EFFECTS OF LOCAL MICROWAVE HYPERTHERMIA AND 5-FLUOROURACIL IN TREATMENT OF EXPERIMENTAL LIVER CANCER.
A Hugander, M Bolmsjö, Lo Hafström and B Gustavsson.
Submitted
- V. MEASUREMENT OF BLOOD FLOW IN RAT LIVER WITH XENON-133.
M Bolmsjö, Lo Hafström, A Hugander and B Persson.
Int J Microcirc Clin Exp 2:27-37, 1983.
- VI. LIVER BLOOD FLOW STUDIES DURING LOCAL HYPERTHERMIA - AN EXPERIMENTAL STUDY IN RATS.
A Hugander, M Bolmsjö, Lo Hafström and B Persson.
Clin Oncol, in press, 1983.

In the text these papers will be referred to by their Roman numerals.

CONTENTS

INTRODUCTION	5
Liver neoplasm and its treatment	5
Historical aspects on hyperthermia	6
Modern hyperthermic treatment	6
Effects of hyperthermia	8
Cellular effects	8
Circulatory effects	8
Effects on the tumor environment	9
Immunological effects	10
The effects on pharmacokinetics	10
Methods for induction of hyperthermia	11
Hyperthermic effects on the liver	13
Hyperthermic effects on liver tumors	14
AIM OF THE PRESENT STUDY	16
MATERIAL AND METHODS	17
Animals and tumors	17
Hyperthermia	17
Tumor size measurements	21
Histopathological examination	21
Enzyme analyses	21
Drug concentration	22
Blood flow measurements	22
Statistics	24
RESULTS	25
DISCUSSION	38
CONCLUSIONS	46
FUTURE ASPECTS	47
ACKNOWLEDGEMENTS	48
REFERENCES	49

"Give me power to produce fever and I will cure all disease"

Hippocrates

INTRODUCTION

Liver neoplasm and its treatment

The liver is the organ most commonly involved in metastases of gastrointestinal cancer. Of the synchronous liver metastases found at laparotomy, 20-25 % are due to colorectal cancer. Approximately 50 % of colorectal tumors will sooner or later give rise to liver metastases (Bengmark & Hafström 1969). Although the incidence of primary liver cancer in Sweden has doubled in the last 15 years, it is still a minor problem with less than 500 new cases every year (Cancer Incidence in Sweden 1979).

The outcome for patients with primary and secondary liver cancer is dismal. A mean survival of one month for primary and six months for secondary cancer is reported (Bengmark and Hafström 1969; Bengmark et al 1971; Bengtsson et al 1981). Liver resection may induce a 2-year survival of approximately 30 % in patients with restricted tumor spread (Almersjö et al 1976). These facts justify an aggressive surgical treatment in selected cases. In patients with scattered metastases in the liver dearterialization and/or chemotherapy has been used but the results are on the whole rather disappointing with a tumor response of 20-30 %. Neither dearterialization nor chemotherapy have induced any significant prolongation of survival (Taylor 1982).

In the search for other means of controlling liver tumor growth several surgeons have been interested in hyperthermia as a complementary mode of treatment (Crile 1961; Parks et al 1978; Storm et al 1979) but few experimental studies have been performed to create a basis for clinical phase-I trials.

Hyperthermia offers a theoretically interesting prospect arising from

its suggested selective effect on tumor tissue, leaving normal tissue intact. For the treatment of non-resectable tumors in the liver this mode of action must be evaluated in experimental and clinical trials.

Historical aspects on hyperthermia

The use of heat for the treatment of different diseases has its origin far back in history. Hippocrates (466-375 B.C.) in Greece introduced the bathing customs of the Egyptians and employed hyperthermia by hot baths for the treatment of insane patients with paralysis. In classic Rome Celsus prescribed sweating baths for pains in the joints, hysteria, dropsy, convulsions and diseases of the kidneys (Giles 1938).

The Japanese in the 17th century were the first to employ artificial fever produced by physical means in the form of Balneo-therapy for the mass treatment of syphilis, arthritis and gout. The body temperature was elevated to at least 39°C in hot water with temperatures between 45-53°C (Giles 1938).

The first "scientific" report on the effect of elevated temperature on tumor tissue was given by W Busch (1866). He described the disappearance of a sarcoma after high fever caused by erysipelas. Coley in 1893 reported successful results after deliberate inoculation of erysipelas in patients with inoperable sarcomas (Coley 1893).

The Swedish gynecologist F Westermarck in 1898 published a report on the beneficial effect of local heating on non-operable carcinomas of the cervix. He used circulating hot water applied to the cervix by means of a metallic loop (Westermarck 1898).

During the early 1900s many investigators reported on excellent tumoricidal effects of heating, but primitive instrumentation and lack of knowledge of hyperthermic effects on the cellular level resulted in uneven results which impeded the evaluation of the hyperthermic treatment.

In an experimental study in 1927, Nils Westermarck was able to demonstrate a selective effect of hyperthermia on tumor tissue at temperatures of 44-45°C (Westermarck 1927). During the next decades tumoricidal effects were convincingly shown in several experimental situations but the clinical interest was only marginal (Johnson 1940; Overgaard and Okkels 1940).

Modern hyperthermic treatment

In 1962 George Crile Jr published experimental studies demonstrating a synergistic effect of heat and irradiation. In transplantable tumors in mice he found a destructive effect of heat beginning at 42°C. He also demonstrated a relationship between temperature level and expo-

sition time; when the temperature was increased by 1°C the same effect of heat could be obtained in half the exposition time (Crile 1962).

In 1967 Cavaliere et al published a review of earlier experiments in the field of hyperthermia and presented their own data showing a selective effect of hyperthermia, both in in vitro and in regional perfusions with heated blood in the extremities (Cavaliere et al 1967).

The biochemical mechanisms of the selective heat sensitivity of tumor cells were further studied by Mondovi et al (1969) and the Overgaards (1972). A possible involvement of cell surface and lysosome membranes was pointed out. These findings along with technological advances in distributing and controlling heat resulted in a renewed interest for hyperthermia in the treatment of cancer.

In clinical practice hyperthermic treatment has been regularly carried out during the last decade. Hyperthermic perfusion with chemotherapeutic drugs in the treatment of tumors localized on the extremities is probably the most widely spread application of hyperthermia (Stehlin et al 1975, Krementz et al 1977, Hafström et al 1982). The value of this type of treatment lies in the salvation of limbs with advanced tumor growth that otherwise would be subjected to amputation. However, no proof of prolonged survival exists, there are no studies yet performed with controls and the therapy seems to have no adjuvant effect in preventing local or regional recurrence (Hafström et al 1982). The definite role of hyperthermia in the regional perfusion system has not yet been established. The results obtained may be attributed to other factors like the regional increase of chemotherapeutic drug concentration, acidosis or hypoxia induced by decreased circulation (Hafström et al 1982).

Hyperthermia has a synergistic effect with radiotherapy, as shown by Crile (1961), Cater et al (1964) and later by Overgaard and Overgaard (1972). The synergism is useful mainly in the treatment of tumors where the sensitivity to irradiation may be decreased or where tolerance to irradiation is decreased by earlier treatment (Hornback et al 1977; Arcangeli et al 1981; Lindholm et al 1982). This combination therapy is being increasingly employed in clinical practice.

In patients with advanced cancer disease, total body hyperthermia has been used as a complement to chemotherapy. Pettigrew reported good tumor responses in a phase-I pioneer study on several types of tumors (Pettigrew et al 1974).

The combination of regional hyperthermia and Imidazole carboxamide (DTIC) infusion has been used in the treatment of melanoma metastases in the liver (Storm et al 1982).

Effects of hyperthermia

Hyperthermia has been defined as a temperature which exceeds the normal body temperature of the animal being studied. In cancer therapy the range of 41-45°C is used. The temperature in a treated tissue must be more than 41°C to be of therapeutical value but must not exceed the tolerance limit of normal tissue (approximately 45°C).

The tumor destructive effect of hyperthermia is multifactorial. The impact of heat on the cell, on the tumor circulation and the tumor environment and on the regional circulation all contribute to the result of the treatment. The different mechanisms depend both on the temperature and the time of exposition (vide infra).

Cellular effects. In vitro and in vivo experimental studies (Overgaard and Overgaard 1972; Giovanella et al 1976). have indicated that cell damage occurs selectively in malignant cells at temperatures between 41 and 43°C while normal cells show no apparent damage under equal conditions at these temperatures. The exact mechanism for this suggested selective effect is not clear and is still under debate (Field and Bleehen 1979). Malignant cells have been shown to have an increased sensitivity to heat in the S and the M phase of the cell cycle (Westra and Dewey 1971). Selective inhibition of tumor cell respiration occurs during heating while the anaerobic metabolism is unchanged (Dickson and Shah 1972). In the cell nucleus heat induces an inhibition of RNA synthesis, both in normal and in malignant cells (Overgaard 1977). Increasing lysosomal activity in the cytoplasm of malignant cells has been observed after heat exposure (Overgaard and Overgaard 1972). The presence of an increased biowater content in the tumor cells has been proposed as another reason for the selective heat sensitivity (Allan and Norman 1974). The involvement of the cell membrane as the primary target was suggested after studies of the surface of heated lymphocytes (Lin et al 1973). A recent hypothesis by Roti-Roti (1982) suggests the following mechanism: heat causes cellular membrane changes that result in increased permeability. Heat also induces protein alterations causing absorption of access protein to chromatin, leading to functional imbalance and, later, cell death.

None of the above mentioned observations can be considered as the single explanation for the heat effect on tumor cells. A multifactorial process is most probable.

Circulatory effects. In normal tissue, heat induces thermal injury with erythema, edema and necrosis as a result. Regulatory mechanisms in the vessels cause increasing vasodilatation and increasing blood flow in the temperature range of 38-45°C. At higher temperatures a cessation of blood flow occurs, probably as a result of vascular damage (Song 1982; Dickson and Calderwood 1980).

Tumor blood flow has been shown to remain fairly constant at

temperatures below 42°C (Gullino 1980; Song 1982; Vaupel et al 1980) whereas at $43-45^{\circ}\text{C}$ vascular stasis and hemorrhage occur, resulting in cessation of the blood flow. Oxygenation and pH in tumor tissue decrease with decreasing blood flow (Vaupel et al 1980).

Different techniques for blood flow measurements have been employed in the above-mentioned studies. The venous outflow method (Vaupel et al 1980) can be used only on strictly isolated tumors. It is far from physiological and therefore not suitable for studies of hyperthermia on tumor and tumor environment. With intraarterially injected microspheres (Song 1982) only the arterial blood flow is registered and the method is therefore less suitable for studies on liver tissue with both arterial and portal circulation. Intravenous administration of isotopes like 86-Rb (Dickson & Calderwood 1980) gives a distribution more in accordance with the true equilibrium distribution but the isotope may be excreted and metabolized during the necessary interval between injection and measurements. Furthermore, these techniques are not repeatable as they include sacrificing the animal before analyses can be performed.

The change in tumor blood flow during hyperthermia is attributed to the construction of the tumor vascular network. This is composed mainly of capillaries formed first from the venous and later from the arterial ends of existing host vessels. These capillaries consist of a single-layered endothelial wall, with no external coat of elastic tissue or smooth muscle, and therefore with no capacity of regulating blood flow. The tumor capillaries are irregularly constricted or dilated and distorted with twistings and sharp bendings. This increases the resistance to blood flow. As the tumor grows the blood flow becomes sluggish because of an elongation of the capillaries causing a decrease in the pressure at their arterial ends. In the periphery of tumors swift circulation may occur due to low tissue pressure and a short capillary length (Song 1982). Even if certain types of tumors have vascularity and a blood flow comparable to that of normal tissue, in most tumors blood flow is lower than the flow rate in the tissue of origin. Furthermore, relative blood flow in most tumors decreases with increasing tumor size. This decrease in tumor blood flow parallels the development of central necrosis in growing tumors (Pettersson 1979).

In summary, the capillary network of tumors and the decrease in tumor blood flow with increasing tumor size predispose for an increased heat uptake in tumor tissue and insufficient dissipation of induced heat, resulting in an overheating of tumor tissue (Crile 1962).

Effects on the tumor environment. The decreased blood flow induced by hyperthermia will cause deficient nutrition, hypoxia and reduction of pH (Kang et al 1982). In tumors with poor vascularization and consequently insufficient exchange between the vascular and the extracellular space, an increased lactate acid accumulation will lead to

increased acidity, which enhances the tumor sensitivity to heat (Overgaard and Bichel 1977).

A decreased deformability of red blood cells due to pronounced acidosis secondary to hyperthermia may impair the tumor microcirculation (Crandall et al 1978; von Ardenne and Reitnauer 1980). This also reduces the oxygen release from the red blood cells to the tissue (Zander and Schmid-Schönbein 1972). The increased acidity decreases the glycolytic rate in tumor cells even if constant arterial glucose concentrations are supplied (Vaupel and Thews 1976). An enhancement of the tumor acidity can be instituted by administration of glucose during hyperthermia which selectively stimulates the aerobic glycolysis of cancer cells, resulting in increased lactic acid formation in tumor tissue (von Ardenne and Reitnauer 1980).

Immunological effects. The immune system probably takes part in the tumoricidal effects that may follow local tumor heating in animals. Macrophages and an increase in non-specific cellular and humoral immune competence may be the mediators for this effect. Total body hyperthermia in animals may depress these functions by the direct heat effect on vital tissues and thereby predispose for enhanced metastases (Dickson and Shah 1980).

In man there is no definite evidence for the stimulation of an effective antitumor response. Neither has total body hyperthermia been shown to impair host defense mechanisms. It is therefore suggested that the effects seen in animals are artefacts due to the greater chemically induced antigenicity of animal tumors (Dickson and Shah 1980).

The effects on pharmacokinetics. The amount of the drug to which tumor cells are exposed in the experimental situation is subject to its distribution, metabolism and excretion. Blood flow changes during hyperthermia may affect the availability of the drug. Oxygen tension and pH of tumor tissue may further influence drug metabolism. In order to investigate the combined effect of hyperthermia and chemotherapeutic agents, therefore, studies must be performed in vivo where all the above-mentioned factors can influence the results.

The combination of heat and an antimetabolite (Methotrexate) was investigated in VX2 carcinoma in rabbits (Muckle and Dickson 1973). This combination was not superior to heat alone, nor was the effect of methotrexate superior to controls. Survival was not affected by combination therapy. 5-fluorouracil, another antimetabolite, was studied in solid Yoshida sarcoma (Dickson and Suzangar 1974) 20 hours after injection of 5-FU (25 µg/kg) hyperthermia at 42°C was administered for 1 hour to a tumor of the foot. No tumor regression was observed.

Adriamycin has been investigated in vivo (Overgaard 1976; Marmor et al 1979). No major improvement on tumor response was observed in these

studies. Magin et al (1980), on the other hand, found adriamycin and local hyperthermia superior to either treatment alone. Variations in drug dosage and application of heat can explain these contradictory results. Potentiation of the tumoricidal effect is described for nitrovin, an alkylating agent (Suzuki 1967), bleomycin (Marmor et al 1979), nitrosurea (Marmor 1979) and melphalan (Honess and Bleehen 1983).

A few pharmacokinetic studies have been performed under hyperthermic conditions. No major changes in distribution and metabolism of adriamycin were induced by whole body hyperthermia at 42.3°C in rabbits (Mimnaugh et al 1978). After local hyperthermia in mouse mammary adenocarcinoma there was an increased retention of adriamycin metabolites in heated tumor tissue when compared with controls (Magin et al 1980). This may be one possible explanation for the improved tumor response. Similar retention of nitrogen mustard was found in rabbit tumors after local hyperthermia at 42°C (Shingleton 1962).

In the treatment of secondary adenocarcinomas of the liver, 5-fluorouracil is a widely used drug with documented tumor-reducing effects in selected cases (Taylor 1982). As the drug is metabolized in the liver, combination with heat may have implications on the pharmacokinetics and the toxicity of the drug. This has not yet been studied in a controlled experimental or clinical situation.

Methods for induction of hyperthermia

In man, the upper temperature limit for total body hyperthermia is 42°C. This can be achieved by hot air or radiant heat (Warren 1935), immersion in hot water (von Ardenne 1972), a space suit with circulating hot water (Larkin et al 1977; Bull et al 1979), immersion in melted paraffin (Pettigrew et al 1974) or perfusion with extracorporeal heat exchange (Parks et al 1978).

Regional hyperthermia in a selected region or part of the body can be given at a temperature of 44-45°C for a restricted time. This has been achieved by immersion in hot water (Crile 1961), radiant heat (Lehmann 1971), perfusion (Cavaliere et al 1967; Stehlin et al 1975), capacitive radiofrequency, (LeVein et al 1976) inductive radio-frequency (Storm et al 1979) and microwaves (Hornback et al 1977).

In local hyperthermia, confined to the tumor and its surrounding tissue, the tumor temperature may reach 50°C. This can be achieved by hot fluid irrigation in cavities (Hall et al 1974), radiofrequency (LeVein et al 1976; Dickson and Shah 1977; Kim et al 1978), ultrasound (Woeber 1965), or microwaves (Mendecki et al 1976).

Ultrasound and electromagnetic heating techniques have attracted the greatest interest in recent years. Ultrasound in frequencies between 0.5-3 MHz can focus energy into small tissue volumes. Absorption is

proportional to the protein content of the tissue. Relatively little energy is absorbed in fat. A great disadvantage of ultrasound is the reflection from tissue interphases resulting in increased heating at the tissue boundaries.

Electromagnetic heating techniques include capacitive, inductive or radiative (e.g. microwave) heating (Fig 1). Capacitive heating is achieved by using flat electrodes. Inductive heating include application of e.g. "pancake" coils for superficial heating as well as placing the patient within a circular coil for heating of segments of the body (Storm et al 1979). Microwave irradiation can be achieved by non-contact antennas (in small animal experimentation) or contact applicators, which are usually used for human subjects.

Frequency

MHz

Wavelength

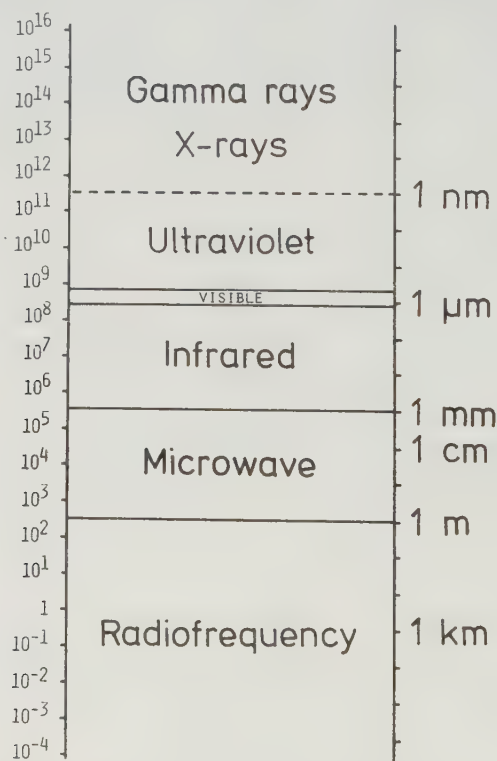


Fig 1. Schematic figure of frequencies and wavelengths of the electromagnetic spectrum.

Two main problems are connected with electromagnetic heating modalities. Contact applicators cause surface heating. This can be avoided by cooling the skin with circulating water or circulating air. Insertion of conducting materials in the energy field may cause an unfavorable temperature distribution with the formation of "hot spots". Hence a thermistor probe, necessary for continuous temperature registration, may register its own "hot spot" temperature rather than the temperature of the surrounding tissue (Baker et al 1978). Non-conductive temperature probes would permit continuous recording of temperatures in the microwave field. Such probes have, however, drawbacks such as large size, instability and high cost.

Microwave irradiation with frequencies of 300-3 000 MHz has been used in the experimental treatment of cancer for the past 30 years. Originally, the equipment was derived from set-ups for physical therapy for muscle and joint dysfunctions. In recent years, however, specially designed equipment has become commercially available. With microwaves, usually at frequencies of 300 to 2 450 MHz, a restricted superficial heating is achieved. However, for studies in small animals the microwave technique is suitable provided that the problems of field interaction and temperature registration can be solved.

Hyperthermic effects on the liver

Under normothermic conditions the temperature of the rat liver and the rat brain is approximately $0.7-1^{\circ}\text{C}$ higher than the intraabdominal temperature. The liver is the most important internal heat source for maintaining the body temperature (Birnie and Grayson 1952). During total body hyperthermia the temperature in the liver remains 0.7°C above intraabdominal temperature, whereas after concluded heating this difference increases, indicating a less efficient heat transfer from the liver (Fletcher et al 1982).

In the in situ perfused rat liver a pronounced decrease in glycolysis and urea synthesis occurs between 42 and 43°C , indicating a marked decline in the liver function. At the same temperature level a decrease in oxygen consumption and increased ammonia production can be registered as criteria for liver injury (Skibba and Collins 1978).

Under similar constant conditions concerning blood flow, oxygenation and pH, isolated perfusion of the rat liver in vitro induced pronounced centrilobular vacuolization at 41°C . A severe dissociation of hepatocytes occurred at 42°C . These microscopic findings were considered to be purely thermal effects. In the same experimental system an increase in the perfusate content of liver transphosphatases (ASAT and ALAT) was found to be time-temperature dependent, occurring earlier at higher temperatures (Bowers et al 1981).

Further support for the sensitivity of the liver to heat was demonstrated in perfused dog livers in vivo, where heating at 43°C for

30 min left no survivors. Forty per cent of the dogs survived at the same temperature if the heating time was reduced to 10 min. In the surviving dogs microscopic analyses revealed a diffuse hepatocyte vacuolopathy progression to ballooning degeneration and later complete resolution (Boddie et al 1979). In this study, technical problems caused 25 % mortality in normothermic perfusion. Six out of ten dogs perfused at 43°C for 30 min died from cardiac arrhythmias which could not be connected with liver injury.

There are few reports concerning the effect of hyperthermia on human liver function. In one patient with advanced malignant disease but without liver engagement, total body hyperthermia at a temperature above 42°C induced parenchymal cell necrosis, cholestasis and jaundice 24 hours after treatment (Wills et al 1976). After total body hyperthermia at a maximum temperature of 41.8°C increased values of LDH and GOT were suggesting hepatic sensitivity to heat (Ostrow et al 1981). Similar findings were reported by Pettigrew et al after total body hyperthermia at temperatures exceeding 41.8°C (Pettigrew et al 1974).

A correlation between the temperature level and increase of liver enzymes seems to exist. Whether the increase in liver enzymes can be taken as an indication of the upper tolerance limit for heating has not yet been established.

The above-described changes in liver functions may be related to variations in the liver blood flow caused by hyperthermia. Few studies have been performed concerning liver blood flow during hyperthermia. Wike-Hooley et al (1981) by means of hepatic vein catheterization found large variations in the clearance of infused indocyanin green indicating hyperthermic effects on liver blood flow.

Experimental methods for studying blood flow in the liver are numerous (Mathie 1982). For repeatable studies in experimental animals there is no suitable method that gives information both on absolute flow values and on relative changes. For studies of liver blood flow during hyperthermia the relative changes are of greater interest than the exact blood flow. It should also be possible to repeat measurements at short intervals.

Hyperthermic effects on liver tumors

Hyperthermic treatment of liver tumors can be of value providing that the blood flow in the tumors is below that of the surrounding parenchyma. This is true for colorectal metastases (Pettersson 1979). The selective effect on tumor tissue makes hyperthermia suitable for the treatment of scattered metastases, as the normal liver parenchyma will be left relatively intact.

Few studies are found in the literature concerning the effects of hyperthermia on liver tumors (Pettigrew et al 1974; Storm et al 1982).

The effect of hyperthermia on tumor growth, development of necrosis and the impact on the surrounding normal parenchyma has not been systematically investigated. No studies have so far been performed with a well-defined tumor system in an experimental situation.

In the clinical treatment of advanced cancer disease, hyperthermia has been used as an adjuvant to chemotherapy. Pettigrew et al (1974) reported good results of total body hyperthermia combined with chemotherapy in six patients with tumor in the liver. Decrease of tumor size, regression of pain and regression of jaundice were described. The liver tumors were of different origins, including hepatic metastases of the colon, cholangiocarcinoma, undifferentiated carcinoma and adenocarcinoma of the gallbladder. In two patients the tumor was not defined. Objective response was found in five out of six patients.

Storm et al (1982) reported tumor regression in 6/10 patients and pain relief in 4/5 patients after regional hyperthermia in combination with intraarterial infusion of DTIC in patients with melanoma metastases of the liver. These results were retrospectively compared with patients with the same disease who were treated with conventional intravenous DTIC. In this group of patients no responders were found. In five patients treated with i.v. DTIC plus regional hyperthermia no response to the treatment was registered. This study can be criticized because of the lack of temperature registrations during treatment. Furthermore, the beneficial effect of the combined treatment might be an effect of decreased arterial supply to the liver, caused by the artery catheter, rather than an effect of hyperthermia.

In the above-mentioned studies the role of hyperthermia versus that of chemotherapeutic drugs is not clarified. To evaluate the possible benefits of hyperthermia in the treatment of liver tumors, hyperthermia has to be applied alone as well as together with other modes of treatment in experimental studies on defined tumor systems and in controlled clinical studies.

AIM OF THE PRESENT STUDY

This study was designed to

develop a technique for hyperthermia, especially suitable for experimental studies on small animals - rats (I).

study the biological and tumoricidal effects of total body hyperthermia (II)

study the effect of local hyperthermia on liver tumors by means of histopathology and tumor growth (III)

study the effect of hyperthermia on the pharmacokinetics and the toxicity of a chemotherapeutic drug (5-fluoruracil) (IV)

develop a technique for repeated studies of hepatic and tumor circulation (V)

study the circulatory changes induced by hyperthermia (VI)

MATERIAL AND METHODS

Animals and tumors

Approximately 500 inbred Wistar rats of both sexes, weighing about 200 g, were used for the experimental studies. The rats were maintained on a standard rat pellet diet and water ad libitum.

During tumor inoculation and tumor size measurements the rats were anesthetized with ether. During local hyperthermia and blood flow measurements the rats were kept under general anesthesia with Nembutal ($6 \text{ mg} \times \text{kg}^{-1}$).

Animals subjected to total body hyperthermia were placed in a special plexiglass cage during the hyperthermia treatment (I, II).

For all experimental series an N-methyl-N-nitrosoguanidine-induced adenocarcinoma (NGW) of the colon was used (Steele and Sjögren 1974). The tumor was propagated intrarenally every 10 days as a rough cell suspension. The condition of the tumor was controlled at regular intervals for occurrence of infection and changes in tumor growth. Syngenicity was demonstrated by the take of the tumor in each generation of the inbred rats. By dissecting the tumor from the kidney, cutting it into small fragments and homogenating by trypsinization, a cell suspension was obtained. The number of vital cells was determined after which the suspension was diluted to a standardized content of viable tumor cells. Through a midline incision the abdominal cavity was opened. 0.1 ml of the cell suspension was inoculated with a thin needle just under the capsule on the ventral surface of the central liver lobe. To minimize leakage of tumor cells at the withdrawal of the needle, a Spongostan sponge was placed over the injection site until hemostasis was obtained. Studies were performed 1-2 weeks after inoculation at a tumor diameter of approximately 10 mm (3-16 mm).

Hyperthermia

Before total body hyperthermia (I, II) the rats were anesthetized with ether. Through a midline incision the thermistor (Yellow Springs Inc, USA, model 511) was placed adjacent to the aorta. The connection core was tunneled subcutaneously to the base of the tail. The rats were placed in a special plexiglass cage with access to water and pellets. This cage was put into a larger one with a constant temperature of 25°C (Fig 2). The thermistors were connected to a computerized control system, which beside recording the temperature also directed the microwave generator (Siemens Radiotherm 305 Magnetron 2.45 GHz with a maximal effect of 200 W). Temperature level and exposition time were preset in the control unit. After recording body temperature for 5 min the microwave generator was automatically turned on for a period of 10 s, alternating with temperature recording periods of 5 s. From the

recorded temperature data the control system could decide whether further heating was required. When the predetermined temperature was reached the generator effect was automatically reduced to avoid overheating.

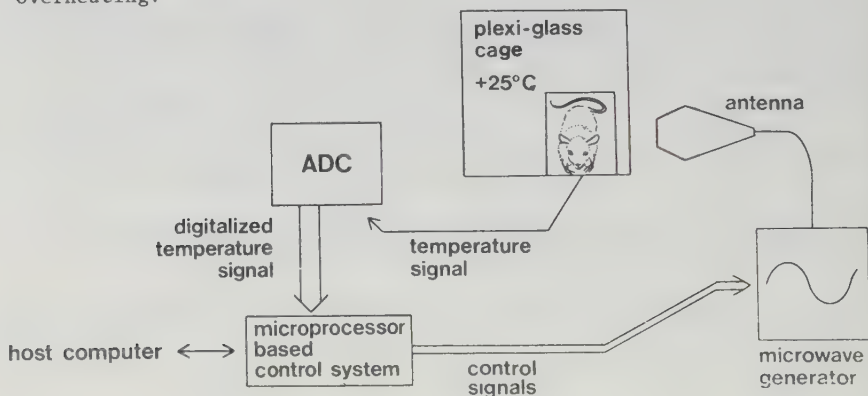


Fig 2. Schematic figure of the experimental set-up for total body hyperthermia in rats.

For local hyperthermia (I, III-VI) the rats were anesthetized and placed on an operation plate kept at a constant temperature by a heating blanket underneath (Fig 3). The tumor-bearing liver lobe (approximately 2-5 g liver tissue) was exteriorized through a midline incision and a master thermistor (Yellow Springs Inc., USA, model 511) was placed in the liver parenchyma opposite the surface in contact with the applicator. In tumor-bearing rats the master thermistor was placed 3-4 mm from the margin of the tumor. Two other thermistors were placed retroperitoneally and in the rectum, respectively. The tumor-bearing lobe with the master thermistor was then covered with an isolating plastic sheet. The microwave applicator (Siemens Focus Radiator 35 mm²) was connected to the tumor-bearing lobe using a transmission gel for improved contact. The thermistors were connected to the ADC and digitalized signals were fed to the computerized control system. The temperature level for the master thermistor was preset at 42.0°C (Fig 4).

In separate studies of the temperature distribution during local liver hyperthermia, beside the master probe another probe was inserted in the center of the liver tumor. In non-tumor-bearing rats another probe was placed 15 mm away from the master probe.

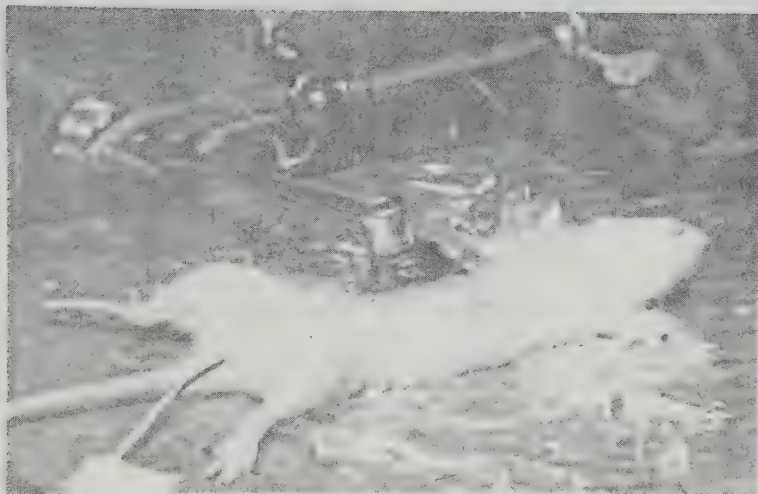


Fig 3. Hyperthermic treatment. The tumor-bearing liver lobe is connected to the microwave applicator using a transmission gel for improved contact.

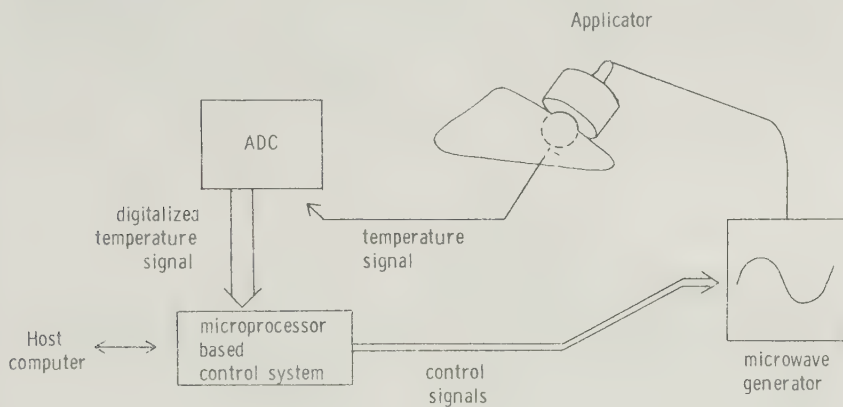
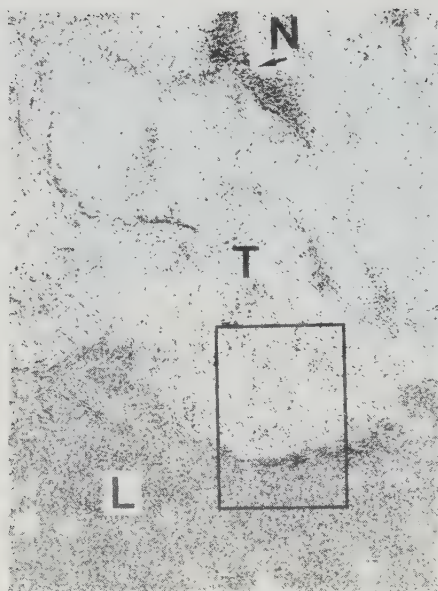
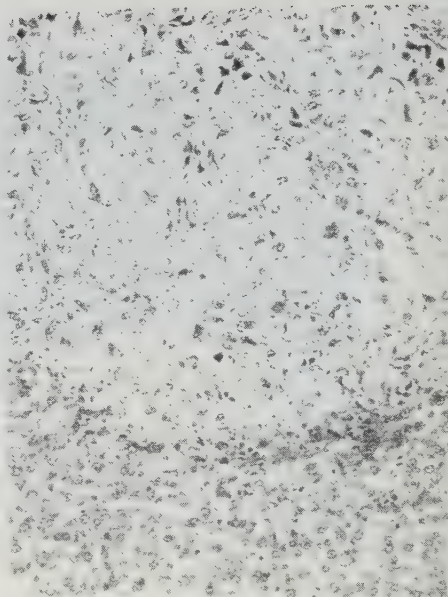


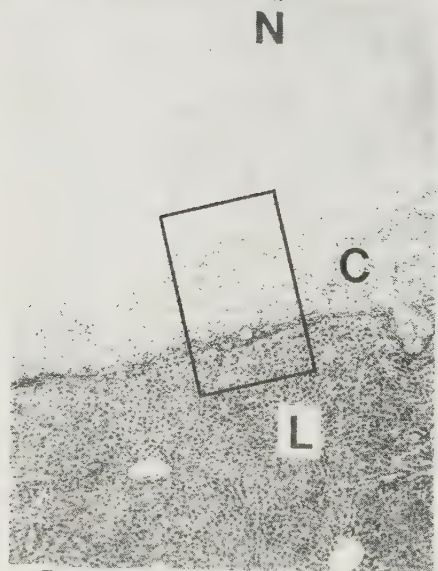
Fig 4. Schematic figure of the experimental set-up for local hyperthermia. The control signals direct the microwave power output to maintain a constant a temperature in the liver lobe.



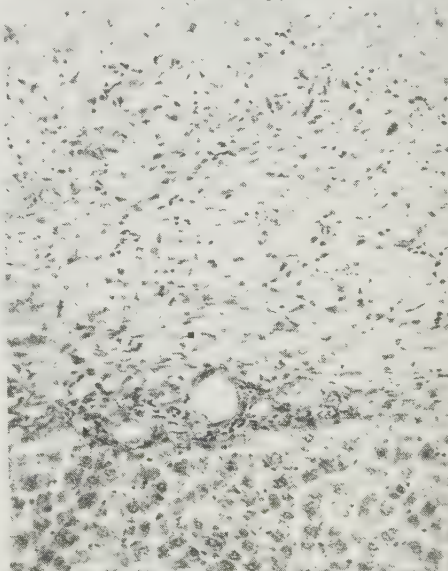
a. Tumor necrosis grade I. x 45.



b. Inset of a. x 180.



c. Tumor necrosis grade VI. x 45.



d. Inset of c. x 180.

Fig 5 a-d. Sections of livers with tumor. L = Liver. C = Connective tissue. T = Vital Tumor. N = Necrotic Tumor.

Tumor size measurements (II, III, IV)

During laparotomy the tumor was measured with vernier calipers on the ventral surface of the central liver lobe. The largest (a) and the smallest (b) visible superficial diameters were recorded. The tumor volume was calculated from these measurements according to the formula (Carlsson 1982):

$$V = a \times b^2 / 2.$$

Histopathological examination (III)

The tumor-bearing liver lobe was excised and fixed in 4 % formaldehyde. One or two slices were cut through the central part of the tumor out to the normal liver tissue. The slices were embedded in paraffin and stained with hematoxylin-erythrosine. The extent of necrosis compared with viable tumor tissue was evaluated in a 6-point scale (1 = minimal necrosis, 6 = most of the tumor tissue necrotic). The investigator was not aware of the treatment being given (Fig 5).

Enzyme analysis (II)

Aspartate-amino-transferase in serum (S-ASAT) was assayed according to the committee of enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Pathology (Scand J Clin Lab Invest 1974). β -hexosaminidase in serum (S- β -nagas) was determined spectrophotometrically (Hultberg et al 1981). For both methods, the within-one-day variation coefficient was below 5 %. Beside the enzyme analyses, erythrocyte volume fraction (EVF) was determined.

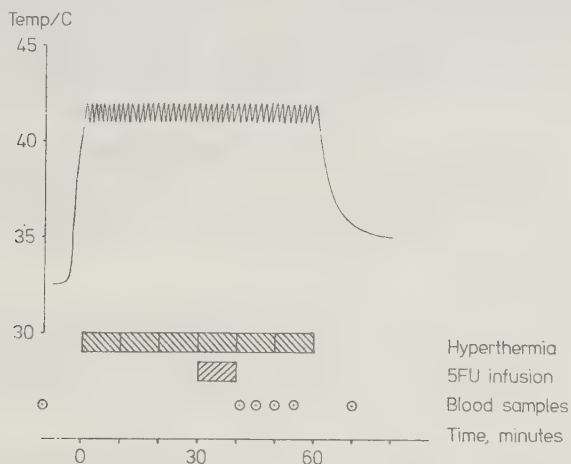


Fig 6. Schematic figure showing time for infusion of 5-FU and time for collection of blood samples during local hyperthermia.

Drug concentration (IV)

5-fluorouracil was given in an i.v. infusion during local hyperthermia in 13 rats. In another 15 rats 5-fluorouracil was infused under normo-thermic conditions (Fig 6). Plasma concentration of 5-fluorouracil was determined by isotachophoretic separation after pretreatment of plasma samples (Gustavsson et al 1979). The reproducibility of the separations was tested with repeated runs using the same samples. The within-one-day variation coefficient was 4 % and the day to day variation coefficient was 7 %.

Blood flow measurements (V, VI)

The blood flow in the rat liver was measured with the ¹³³Xenon clearance technique. For evaluation of the method three routes for administering the isotope to the liver were employed: injection via the portal vein, via the hepatic artery and directly into the parenchyma. For portal injection (V, VI) a catheter with an outer diameter of 0.63 mm was introduced through a distal branch of the superior mesenteric vein. For arterial injection (V) a polyethylene catheter with an outer diameter of 0.61 mm was inserted into the gastroduodenal artery and placed with the tip just caudal to the origin of the hepatic artery. Intraparenchymal injection of 0.1 ml isotope solution was performed directly into the parenchyma (V, VI). The injection site was covered with Spongostan sponge to absorb probable leakage.

A CdTe (cadmiumtelluride) miniature detector with an energy resolving capacity was provided with a cylindrical lead collimator (1 cm diameter) and mounted close to the exteriorized liver lobe. In rats with closed abdomen (V) the detector was mounted to cover the entire liver. Overlapping from other regions was minimized. The detector was connected to a data acquisition system which accumulated and stored the incoming impulses in 2 s intervals. The wash-out curves were registered for 20-25 min from the moment of injection.

For blood flow studies during local hyperthermia at 42°C (VI) the intraportal and intraparenchymal routes of injection were used. The wash-out curves obtained after injection of ¹³³Xenon were analysed by using a bi-exponential approach according to the equation

$$A(t) = P_1 \cdot \exp(-k_1 \cdot t) + P_2 \cdot \exp(-k_2 \cdot t)$$

where $A(t)$ is the Xenon activity in the organ at time t . P_1 and P_2 are the initial amounts of Xenon in each compartment, respectively, and k_1 and k_2 are the elimination rates of Xenon from the two compartments, respectively (Andersen and Ladefoged 1967).

This equation was fitted to the experimental wash-out curves by using the method of least squares. The computer algorithm used to perform the curve analysis was based on the Taylor series linearization

technique and coded in FORTRAN, a technique commonly used when fitting non-linear equations to experimental data (Draper and Smith 1966).

Repeated analyses of each wash-out curve were performed with a successively increased start time of the fit. The start times ranged from time zero (the time of injection) to 3 min later with 10 s increasing start time between each new calculation. The mean value of the fastest component, k_1 , from the computations with start times between 1 and 2 min, was taken as the index of the liver blood flow. The spread in k_1 , from the repeated analyses, was taken as a figure of the precision of the blood flow determination. If the clearance of Xenon was exactly biexponential, the repeated analyses should give the same results, whereas an erroneous model would be detected by diverging results. Differences in the results by using different end-points in the curve fitting were also used to indicate possible failures of the bi-exponential model (Fig 7).

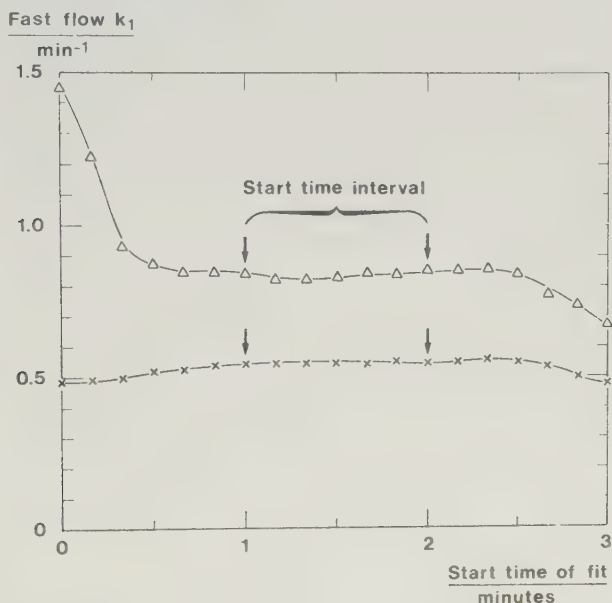


Fig 7. Two typical registrations of k_1 using different start times at the mathematical analysis. The rapid decline of k_1 (upper curve) during the first minute of washout was usually observed. In other cases (lower curve) k_1 was constant and independent of the start time of the analysis. The mean value of k_1 obtained from the computations with start times between 1 and 2 min after the injection was used as the index of the blood flow.

At repeated studies small amounts of Xenon could remain in the tissues due to previous injections. It was assumed that this activity reflected the clearance from the slower compartments and it was not included in further calculations.

Statistics

The following statistical methods were used in the study: Student's t-test for paired and unpaired observations (I, II, IV, V), analyses of covariance (II) and Pitman's non-parametric two-tailed permutation test (IV, VI).

RESULTS

With the applied technique of whole body hyperthermia, heat was effectively and rapidly distributed throughout the whole rat. No localized overheating of the skin was observed. In the central part of the rat the temperature was stable around the preset level. A regulating precision of $\pm 0.1^{\circ}\text{C}$ was maintained at the master probe position in all runs. The difference between the intraabdominal and intrarectal temperature was less than 1°C while the temperature in the superficial layers of the body was approximately 1°C lower as a result of heat exchange with the surrounding air. The time delay between the microwave switch-off and temperature recording was not critical for the effectiveness of heating (Fig 8 a).

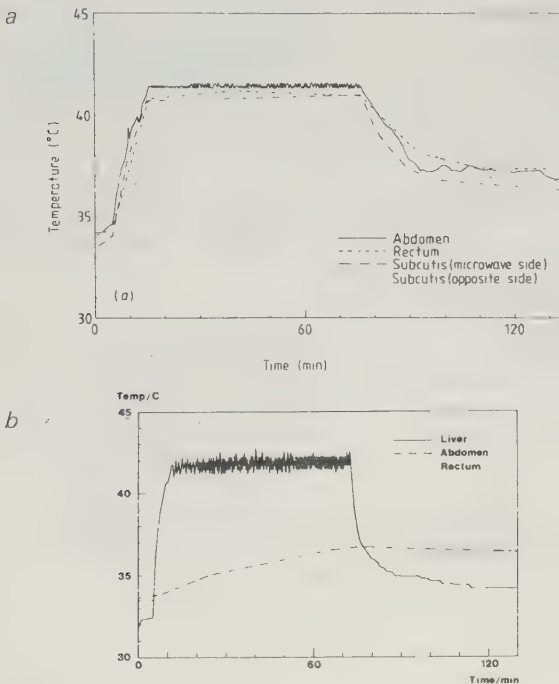


Fig 8. Temperature profiles during (a) total body hyperthermia and (b) local liver hyperthermia. Preset temperature was 41.5°C in (a) and 42°C in (b).

Local hyperthermia caused mean temperature variations of up to 1.0°C from the preset temperature level, with rapid fluctuations of maximum $\pm 2^{\circ}\text{C}$ (Fig 8 b). Temperature variations of $\pm 2^{\circ}\text{C}$ were found in normal liver tissue that exceeded a volume of 1 cm^3 . The temperature in the tumor correlated well with that in the liver parenchyma and was $2\text{--}6^{\circ}\text{C}$ higher, depending on the size of the tumor (Fig 9).

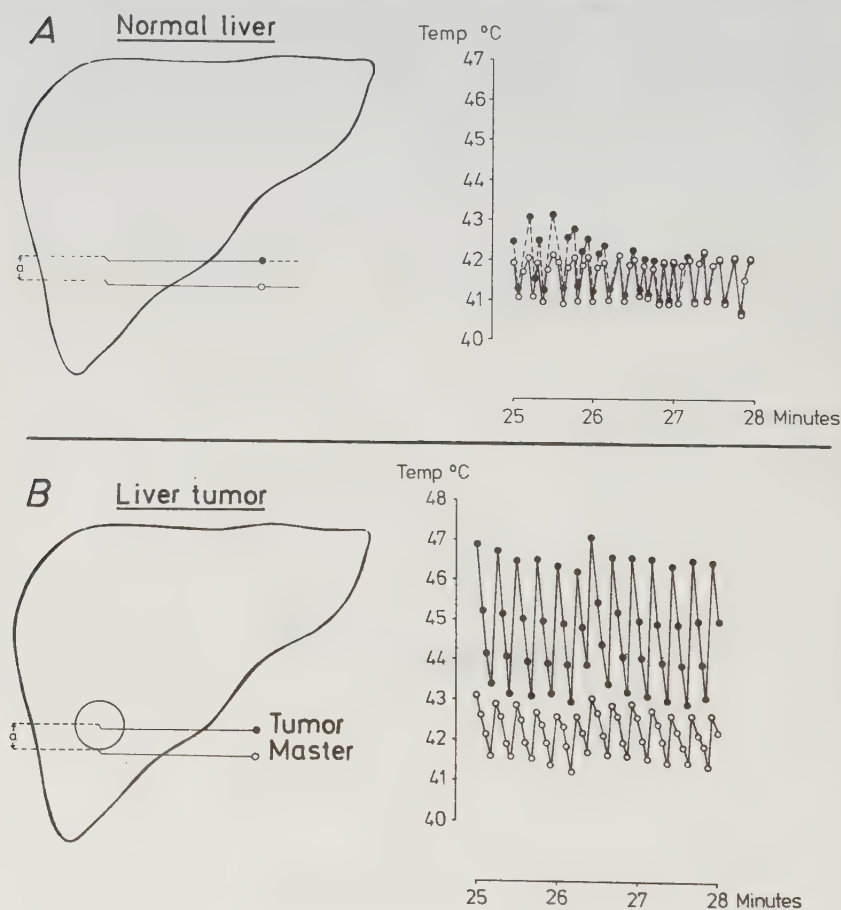


Fig 9. Temperature curves demonstrating the selective "over-heating" in tumor tissue (A) normal liver parenchyma, (B) the master thermistor located in normal liver parenchyma close to the tumor. Another thermistor located in the center of the tumor.

Local hyperthermia of the liver caused only a minimal increase in intraabdominal and rectal temperature (Fig 8 b).

Mortality (II, III, IV)

After a single exposure to total body hyperthermia at 41.0°C all rats survived. At 41.5°C all rats survived 1 h exposure while 2 h exposure resulted in the death of 2/6 animals. At 42°C 5/6 rats died after 1 as well as 2 h exposure (Table I).

Table I. Survival of rats after one exposure to total body hyperthermia at different temperatures.

Temperature	Duration hours	Survival > 100 days
41.0	1	6/6
41.5	1	6/6
42.0	1	1/6
41.0	2	6/6
41.5	2	4/6
42.0	2	1/6

When hyperthermia was given at 8 h intervals during a 24 h period no mortality was registered at 41.0°C. After 1 h exposures at 41.5°C 3/8 rats died, at 42°C all rats died. Two hour expositions were performed in 8 rats at 41.5°C, no rats survived. All deaths occurred within 2 days after treatment (Table II).

Table II. Survival of rats after repeated exposures to total body hyperthermia at different temperatures.

Temperature	Duration hours	Survival > 100 days
41.0	3 x 1	8/8
41.5	3 x 1	5/8
41.5	3 x 2	0/8
42.0	3 x 1	0/8

At local hyperthermia of 42°C for 1 h 3 of 28 rats died, all of them during treatment due to technical errors (III). When hyperthermia was combined with infusion of 5-FU 4/13 rats died within the first week (Table III). One of these rats died on the day of treatment. All rats infused with 5-FU under normothermic conditions survived the first week. When blood samples were drawn from rats treated with 5-FU and hyperthermia 4/10 died within 60 min whereas 1/10 rats not subjected to hyperthermia died (IV).

Table III. Mortality during the first two weeks after treatment with 5-FU, 5-FU + local hyperthermia and control.

	n	Mortality 1st week	Mortality 2nd week
5-FU + HT	13	4	4
5-FU	15	0	3
Control	11	0	5

Effects on liver enzymes and lysosomal enzymes (II)

Significant increases of S-aspartate-amino-transferase (ASAT) and S- β -hexosaminidase (β -nagas) were seen after total body hyperthermia at 42.0°C. At 41.5°C no significant increases were registered. An increase was also seen in EVF during treatment, which however, was not statistically significant (Fig 10).

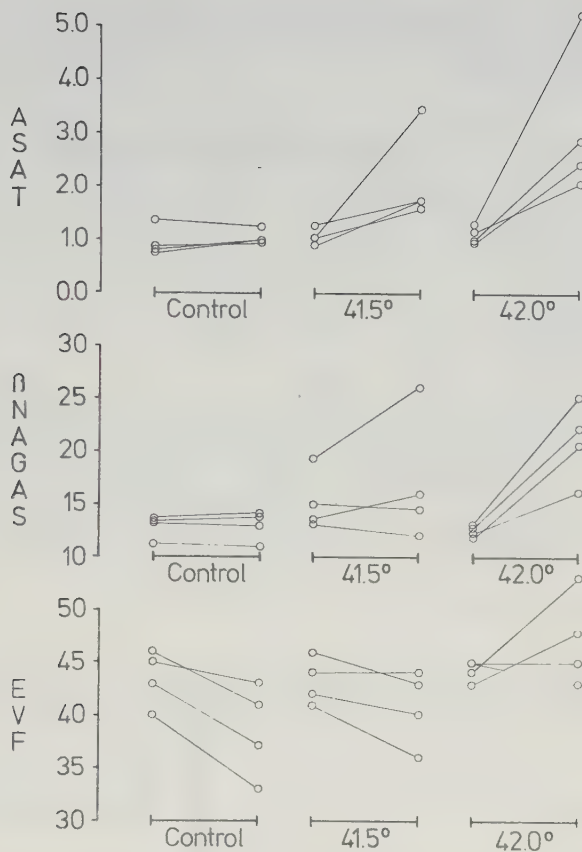


Fig 10. Diagrams illustrating values of S-ASAT ($\mu\text{kat/l}$), β -nagas (u/l) and EVF (%) in control animals and animals subjected to hyperthermia of 41.5°C and 42°C , respectively, during 1 h.

Effects on tumor growth (II, III, IV)

Total body hyperthermia at 41.5°C for 1 h three times during a 24 h period caused no significant difference in tumor growth when compared with controls 3 and 10 days after treatment (Table IV).

Table IV. Liver tumor volume after total body hyperthermia 41.5°C one hour three times in a 24-hour period.

	n	Tumor volume (mm ³)	
		3rd day	10th day
Hyperthermia	11	15.8	455
Control	17	50.0	545

Local hyperthermia at 42°C for 1 h induced a statistically significant reduction in tumor growth 7 days after treatment, when compared with controls ($p < 0.001$). The most pronounced effect was observed in tumors with volumes of 300-500 mm³ (Fig 11, Table V). A statistically significant difference in the development of necrosis was found between treated animals and controls ($p < 0.01$). Necrosis was most pronounced in tumors with a volume of more than 900 mm³ (Fig 12, Table VI).

Tumor volume at day (H + 7)
mm³

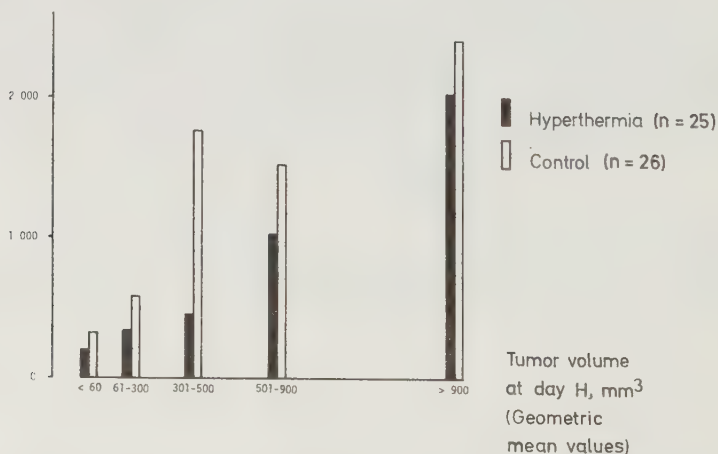


Fig 11. Geometric mean tumor volumes 7 days after local hyperthermia (filled staples) and controls (open staples).

Table V. Geometric mean tumor volume 7 days after local hyperthermia and control.

Tumor volume at treatment (day H)	Tumor volume day H + 7			
	Hyperthermia	n	Control	n
0 - 60	195	4	321	6
61 - 300	341	7	585	4
301 - 500	451	3	1 759	5
501 - 900	1 025	7	1 514	5
> 900	2 023	4	2 398	6
		25		26

Table VI. Tumor necrosis at autopsy. Median values for the extent of necrosis evaluated on a scale from 1 (minimal necrosis) to 6 (most of tumor tissue necrotic).

Tumor volume at treatment (day H)	Tumor necrosis at autopsy			
	Hyperthermia	n	Control	n
0 - 60	2	4	2	6
61 - 300	3.5	6	2	3
301 - 500	4	3	3	4
501 - 900	5	7	4	3
> 900	6	3	3	5
		23		21

Tumor necrosis

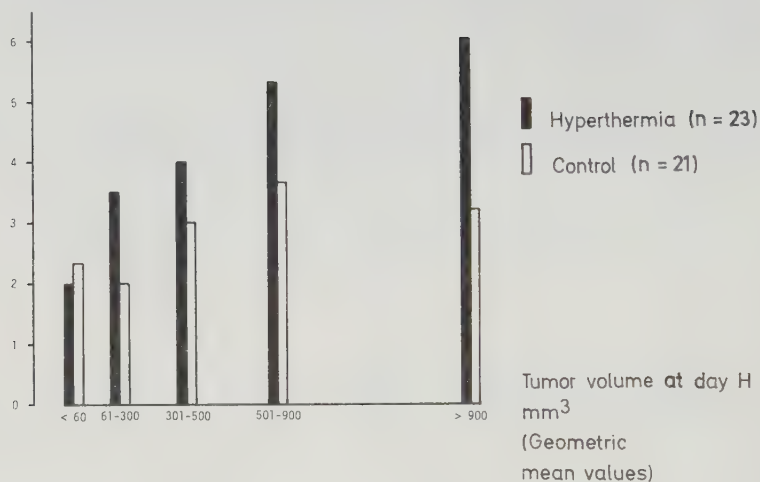


Fig 12. Necrosis in liver tumors of different volumes exposed to local hyperthermia (filled staples) and controls (open staples). The extent of necrosis is described in a 6-point scale.

In rats subjected to local hyperthermia and 5-FU infusion, tumor growth was significantly reduced in comparison with that of rats infused with 5-FU under normothermic conditions ($p < 0.05$) and untreated controls ($p < 0.05$) (Table VII). The effect of hyperthermia + 5-FU was not found to be superior to the effect of local hyperthermia alone (Fig 13) when comparison was made with liver tumors of corresponding sizes only subjected to hyperthermia (III).

Table VII. Differences in tumor volume seven days after local hyperthermia treatment (mean values $\pm$ SD). p-values for comparison between the three groups are given in the table.

	Tumor volume difference (mm ³) (volume H + 7) - (volume H)	p-value
5-FU + H	580 $\pm$ 490	-
5-FU	1310 $\pm$ 920	0.029
Control	1440 $\pm$ 880	0.018

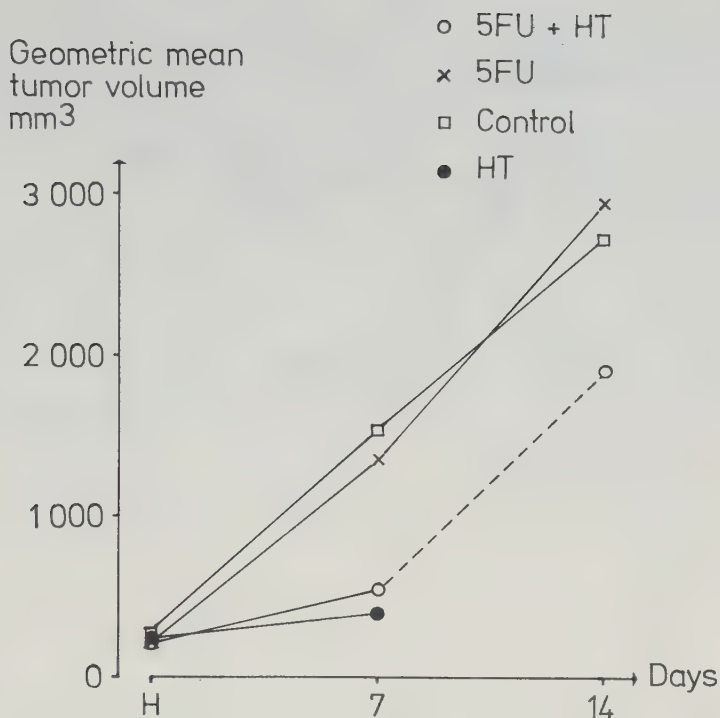


Fig 13. Geometric mean tumor volumes (mm^3) measured on the day of treatment (H), 7 and 14 days after 5-FU infusion with or without hyperthermia. For comparison results of hyperthermia alone in 6 rats are plotted (II).

Effects on pharmacokinetics of 5-FU (IV)

When 5-FU was infused under normothermic conditions a bi-exponentially declining concentration time curve was obtained. The $t_{1/2}$ value for the first phase (α -phase) was 4 min, for the second phase (β -phase) 19 min. During local hyperthermia the declining plasma concentration was mono-exponential with a $t_{1/2}$ value of approximately 5 min.

The area under the concentration curve from zero to the last sample (AUC) was significantly larger for the non-hyperthermic group ($p < 0.05$) (Fig 14).

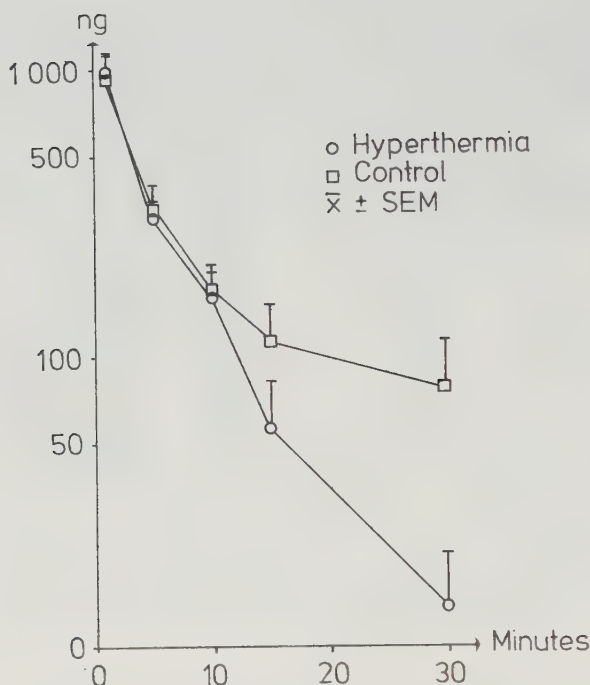


Fig 14. Plasma concentration time curves (mean values $\pm$ SEM) after administration of 20 mg 5-FU/kg body weight as intravenous infusion during 10 min. First sample collected 1 min after end of infusion.

Results of blood flow measurements (V, VI)

Intraportal and intraarterial injection of $^{133}\text{Xenon}$ gave mean values of k_1 of $0.52 \pm 0.19 \text{ min}^{-1}$ and $0.51 \pm 0.15 \text{ min}^{-1}$, respectively ($0.36 \text{ ml} \times \text{min}^{-1} \times \text{g}^{-1}$ if converted to specific blood flow). Injection of Xenon directly into the liver parenchyma gave k_1 values of $0.80 \pm 0.33 \text{ min}^{-1}$ ($0.56 \text{ ml} \times \text{min}^{-1} \times \text{g}^{-1}$). The difference in k_1 between intraparenchymal injection and portal and arterial injection, respectively, was statistically significant ($p < 0.001$, Student's t-test) (V). The intraindividual spread in k_1 was 27 % for portal, 25 % for arterial and 18 % for intraparenchymal injection ($\pm 1\text{SD}$ around the individual mean value) (Table VIII). k_1 values were significantly higher in animals with the liver in the natural position compared to animals with exteriorized liver ($p < 0.001$). The use of successively increased start-times for the numerical analyses of the wash-out curves was found to be valuable in estimating the accuracy of the results.

Table VIII. Summary of the results from the calculations of the liver blood flow in rats (V).

	Administration technique		
	Portal vein	Hepatic artery	Parenchymal
No of animals	8	7	10
No of experiments	31	22	24
Mean flow index, k_1	0.52 ± 0.19	0.51 ± 0.15	0.80 ± 0.30
Intra-individual spread (1 S.D.)	27 %	25 %	18 %
Average uncertainty in k_1	7.4 %	9.5 %	5.6 %

Table IX. Mean values of k_1 for the different techniques of administration of Xenon during normothermic and hyperthermic conditions (VI).

Xenon ¹³³ administration	Tumor	n	Prehyp	Hyp	Posthyp
Portal	No	6	0.52 ± 0.13	0.39 ± 0.17	0.57 ± 0.14
Portal	Yes	6	0.59 ± 0.18	0.50 ± 0.23	0.53 ± 0.05
Intraparen- chymal	No	5	0.66 ± 0.24	0.57 ± 0.19	0.70 ± 0.13
Intratumoral	Yes	4	0.60 ± 0.26	0.48 ± 0.21	0.50 ± 0.14
Total		21	0.59 ± 0.19	0.48 ± 0.20	0.58 ± 0.13

A decrease of k_1 was observed during local hyperthermia (0.59 - 0.48 ($p < 0.05$)) (Table IX, Fig 15). After hyperthermia the blood flow returned to pre-hyperthermic levels in all groups except in the group where blood flow was measured via an intratumoral injection. The post-hyperthermic blood flow in this group differed significantly from blood flow in normal parenchyma, measured by an intraparenchymal injection ($p < 0.05$) (Fig 16).

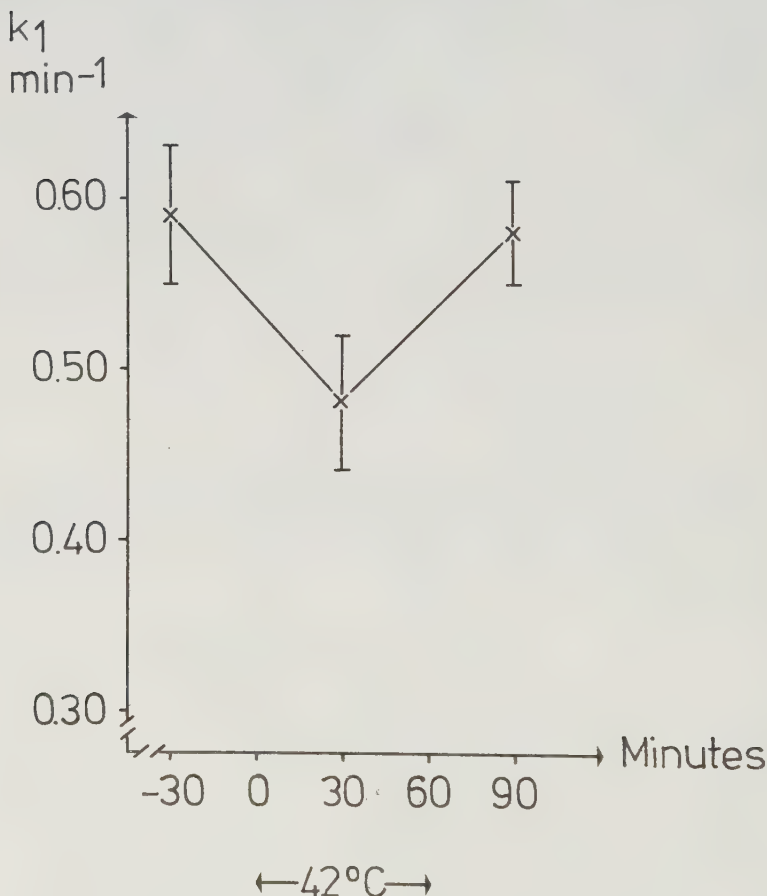


Fig 15. Variations of k_1 during hyperthermia. Blood flow measurements performed by portal and intraparenchymal administration in normal liver tissue and in tumor ($n = 21$). Mean values $\pm$ SEM.

k_1
 min^{-1}

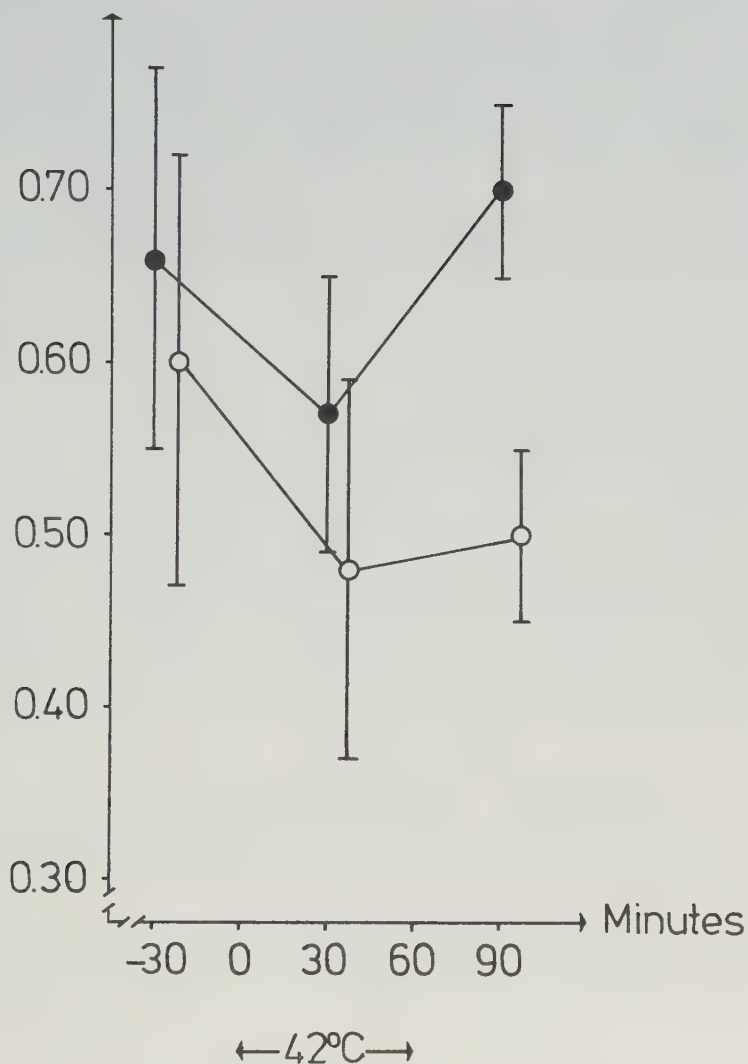


Fig 16. Variations of k_1 during hyperthermia, $\bullet$ = intraparenchymal administration, $n = 5$; $\circ$ = intratumoral administration, $n = 4$. Mean values $\pm$ SEM.

DISCUSSION

Studies of hyperthermia in small laboratory animals like Wistar rats are important for a better understanding of the effects of hyperthermia in biological systems. In vitro studies certainly give information on the cellular level but do not include the physiological mechanisms involved. Studies in larger laboratory animals like dogs and pigs have their draw-backs of high costs as well as the lack of a reliable tumor system for studies on tumor responses. Clinical studies naturally offer the best biological situation but the heterogeneity of tumor type and spread, concomitant cytostatic treatment and ethical aspects of control material restrict a proper clinical evaluation of hyperthermic effects.

The technique of inducing liver tumors by inoculation of a defined tumor cell suspension directly into the liver parenchyma has been found to be highly reproducible and give solitary, well-defined ellipsoid liver tumors. The tumor system used in this study has been previously investigated concerning arterial blood flow (Sundqvist 1980) and growth characteristics (Carlsson 1982).

Microwaves: Experimental work with microwaves in the range of 300-3 000 MHz in tumor therapy was reported in the 1950s (Gessler et al 1950; Allen 1955). A variety of animals and microwave frequencies were used in experimental trials (Cater et al 1964) but these lack details concerning tumor types, temperature in the tumor, duration of heating etc making much of this work difficult to evaluate. Furthermore, the heat was often employed to enhance the effects of ionizing radiation or drugs. Dietzel et al (1975) used microwaves alone for the treatment of Erlich ascites tumors in mice. In this and most of the other studies from this period the problem of temperature recording was not satisfactorily solved.

In patients microwave hyperthermia has been used in combination with x-rays for treatment of different kinds of tumors. A synergistic effect on tumor growth has been achieved by combining hyperthermia with conventional radiation therapy in clinical studies (Hornback et al 1977; Lindholm et al 1982).

The low penetration depth of microwaves in the range 300-2 450 MHz restrict their use to superficial tumors within a depth of about 3-4 cm. By using radiofrequency heating at lower frequencies (30-100 MHz) and multiple applicators even deepseated structures can be heated (Short and Turner 1980).

In the present study on total body hyperthermia (I, II) the superficial tissue of the rats quickly absorbed the heat. Since the penetration ability of 2 450 MHz microwaves is poor, most of the energy was absorbed superficially at a depth of less than 1 cm. The heat was then quickly distributed throughout the body by the blood circulation.

This may explain why no overheating of superficial structures occurred.

For studies on local hyperthermia (I, III, IV, VI) a cigar-shaped heat distribution pattern of 1-2 cm in diameter and a depth of 1 cm was sufficient for the relatively small volume of liver tissue treated during local hyperthermia. Approximately 5 g of liver tissue was subjected to hyperthermia. This explains the temperature variations of $\pm 2^{\circ}\text{C}$ that were observed during heating.

By alternating microwave irradiation with temperature recording the problem of field interaction of the thermistor probes was avoided. The technique proved to be appropriate and simple for obtaining controlled hyperthermia. The microprocessor based control system allowed an automatically performed series of single and repeated hyperthermia exposures, involving manual procedures only at the start and the end of the experiments.

A theoretical development of the hypothesis (I) concerning pulsed microwave thermometry has recently been published by Losheck (1982). He suggests that even shorter periods of temperature registration may be preferable to avoid rapid cooling of locally heated tissue, as found in the present study (II, III, IV). For clinical use this cooling is of no importance since the heated tissue volume is larger than in the experimental situation.

Tolerance to hyperthermia: During total body hyperthermia the maximal tolerated intraabdominal temperature for rats was found to be 41.5°C , regardless of the number of exposures (II). In humans survival has been reported after rectal temperatures as high as 44.4°C (Ballard 1974). In animals the liver function has been studied at increased temperatures as the liver is considered to be a critical organ when the body temperature is elevated (vide infra).

In the present study (II) an increase of temperature from 41.5°C to 42°C caused a marked decrease in survival, corresponding to a significant increase in S-ASAT as a sign of liver cell injury, and a significant increase in S- β -nagas reflecting cell damage or increased phagocytic load on the RES (Sieck et al 1977). The critical limit for perfused rat liver at $42-43^{\circ}\text{C}$ as described by Skibba and Collins (1978) corresponds well with the present findings (II) as there is always a constant difference of 0.7°C between the liver and intra-abdominal temperature (Birnie and Grayson 1952; Fletcher et al 1982).

With isolated hyperthermic perfusion of liver tissue in rats a higher tolerance level was found (Brauer et al 1963; Skibba and Collins 1978). However, perfusion in an isolated system includes a maintained constant blood flow during the procedure and physiological variations in blood flow during hyperthermia will not be considered in the results.

A central role of tumor cell lysosomes in the effect of heat on tumor cells was suggested by Overgaard and Overgaard (1972). They proposed that a release of lysosomal acid hydrolasis in tumor tissue was the most likely explanation for the destructive action of heat treatment alone, as well as in combination with roentgen. No changes were seen in normal cells. In later experiments Overgaard (1976) found minor reversible changes in non-malignant cells after hyperthermia at 43°C, with just a slight increase in the amount of lysosomes. A rise in acidity of tumor cells would be one explanation for the increased outflow of lysosomal enzymes (Overgaard and Overgaard 1972). An increased fragility of lysosomes in neoplastic cells would be another reason for the selective cell destruction of heat (Turano et al 1970). In the present study there was a release of lysosomal enzymes from normal tissue after total body hyperthermia at a temperature of 42°C (II). This increase in serum lysosomal enzyme activity paralleled with an increased mortality of the experimental animals. As these animals had no tumors, the lysosomal enzyme originated from normal tissue, probably the liver. As mentioned above, the increase in β -hexosaminidase may also reflect an increased phagocytotic load on the RES.

Effect on tumors: Total body hyperthermia at 41.5°C, 3 times in a 24 h period, induced no significant effect on the growth of liver tumors (II). In a previous preliminary study a minor reduction of tumor growth was noticed 3 days after treatment, but disappeared 7 days later (Hafström et al 1979). In another study (Carlsson et al 1983) total body hyperthermia in combination with hepatic artery ligation had an additive effect on tumor growth. In the mentioned studies, as well as in the present one (II), the effect of heat was not investigated by means of histopathological examination. Edema and swelling of the tumors may disguise a difference after treatment. Another explanation to the lack of effect may be that total body hyperthermia does not induce a sufficient heat in the liver tumors.

The effects of local hyperthermia on implanted tumors have been thoroughly investigated in superficial tumors located in subcutaneous tissue and muscle, mainly on the extremities. Local heating is easily achieved and intratumoral temperature registration can be performed by puncturing of the tumor without the risk of spreading tumor cells to surrounding organs. Tumor growth can be registered by daily measurements. This enables administration of heat in relation to a given tumor volume. The effect of the treatment is easily evaluated. Repeated exposure to hyperthermia can be given until tumor regression is achieved.

For tumors in the rat liver hyperthermia treatment involves technical and methodological problems. With only invasive techniques available registration of tumor growth must be performed at laparotomy. Repeated laparotomies may impose on the tumor-host relationship and accelerate tumor growth (Fisher and Fisher 1959). Furthermore, the trauma of repeated narcosis and laparotomies may influence the survival of the experimental animals.

Whereas temperature registration in superficial tumors can be performed with intratumoral temperature probes, the puncture of a liver tumor may cause dissemination of tumor cells into the abdominal cavity, thus influencing the experiment in an unfavorable manner. This problem was avoided by insertion of the thermistor probe in normal parenchyma, adjacent to the tumor, thus avoiding overheating of the normal parenchyma. This technique will be reliable, provided that selective heating occurs in the liver tumor as was demonstrated in the present study (I, Fig 9).

For registration of tumor growth CT scan and angiography would be theoretically possible (Carlsson et al 1982) but in the experimental situation these techniques would be too crude and inexact.

In spite of these difficulties it is essential to perform in vivo studies of hyperthermia effects on the liver if the results are to be used as a basis for future experimental and clinical trials.

The selective heating of tumor tissue, proposed for other locations (Crile 1962) was found to be even more pronounced in the treatment of liver tumors. A difference of $2-6^{\circ}\text{C}$ was obtained between normal parenchyma and tumor tissue. The difference between 2 points of measurement in normal parenchyma usually did not exceed 1°C . As a result of this selective heating microscopic signs of necrosis and fibrosis occurred in the surrounding parenchyma of large tumors, probably as a result of the high temperature in the tumor (III). Apart from the histopathological examination, the possibility of minor liver cell damage in the tumor-bearing lobe has not been investigated.

The technique used for the evaluation of tumor volume and tumor growth rate has been employed often and is considered to be highly reproducible (Carlsson 1982). Tumor effect was evaluated by calculation of tumor volume. A significant reduction of tumor growth was registered in small and medium-sized tumors, while the effect was less obvious in larger ($> 900 \text{ mm}^3$) tumors. The effect on small tumors in the present study differs from studies of subcutaneous adenocarcinomas (Slotman et al 1980) and subcutaneous sarcomas (Auda et al 1980) of similar sizes. In these studies no effect of heat was obtained in small tumors. The difference may be explained by a better selective heating in parenchymatous tissue than in subcutaneous tissue, where the cooling effect of the surrounding air reduces the heat obtained in the periphery of the tumor.

A decrease in relative tumor blood flow with an increase in tumor size has been demonstrated by Shibata and MacLean (1966) for experimental tumors in subcutaneous tissue. A similar decrease in the arterial blood flow was demonstrated in small adenocarcinomas of the liver of Wistar rats (Sundqvist et al 1978). This predisposes for a more pronounced effect of hyperthermia in larger tumors because of a reduced dissipation of heat resulting in an "overheating" of tumor tissue. In

superficial tumors this increased effect will be seen as a shrinkage of tumor tissue and a reduction of tumor size within a few days. In the dense liver parenchyma the registered volume of treated tumors may not differ from control tumors and hence the tumoricidal effect of treatment may be disguised. An evaluation of necroses in the present study gave complementary information and the pronounced effect on larger tumors was clearly demonstrated. This is in good agreement with observations by Storm et al (1979), who found that in human liver tumors vascular necrosis and thrombosis prevented absorption of the destroyed tumor and allowed only fibrous replacement with little change in tumor size.

Effect on 5-fluorouracil: Studies concerning the combined effect of hyperthermia and 5-FU were performed in rats with medium-sized liver tumors (approximately 300 mm³) (IV). The dose of 5-FU (20 mg x kg⁻¹) was chosen with the purpose of administering a dose that in itself was considered to have a low immediate toxicity. Administration of a single dose was not expected to have any significant tumoricidal effect, but was mainly performed for toxicological and pharmacokinetic studies. No difference in tumor growth was observed in rats treated with 5-FU under normothermic conditions, compared with controls. The growth retardation after combined treatment (hyperthermia and 5-FU) was not superior to hyperthermia alone (III).

Based on in vitro studies Paltzer and Heidelberger (1973) have proposed that drugs inhibiting DNA and protein synthesis like 5-FU might protect cells against hyperthermic damage. Mizuno et al (1980) found no potentiation of toxicity when combining 5-FU with hyperthermia in cultured cells. In vivo studies by Dickson and Suzangar (1974) showed no effect of 5-FU on Yashida sarcoma, neither at 37 nor 42°C local hyperthermia. However, in that study hyperthermia and drugs were administered with a 20 h interval and gave no information of a probable synergistic effect. Furthermore, the tumor was classified as undifferentiated and had no sensitivity to 5-FU.

In the present study an obvious negative effect of combined treatment was the increase in mortality during the first week after treatment (IV). Four out of 13 rats died after 5-FU + hyperthermia, whereas all rats survived 5-FU infusion (Table IV). After local hyperthermia alone 3/28 rats died, all of them during treatment due to technical errors (III).

The increased mortality after thermochemotherapy may be caused by an enhanced drug toxicity, of which the mechanism is not clarified. Hyperthermia may cause a faster distribution of the drug, i.e. more of the drug reaches the liver, where uptake and metabolism is accelerated. On the cellular level, changes in membrane permeability and effects on the enzyme systems may alter the metabolism of the drug, thus causing increased drug toxicity. In vitro a decrease in the anabolic pathway of 5-FU occurred in tumor cells during hyperthermia

(Kummer and Kraml 1977). This would theoretically cause a prolonged second-phase in the normal bi-phasically declining plasma concentration curve of 5-FU. Only 5 % of administered 5-FU is anabolized in vivo, therefore changes in anabolic pathways are of minor importance. In the present study with selectively increased temperature in the liver and only a slight increase in blood temperature, the accelerated decline in plasma concentration during hyperthermia may reflect accelerated catabolism.

Daly et al (1982) studied the effect of intraarterial infusion of 5-FU ($10 \text{ mg} \times \text{kg}^{-1}$) in dogs during systemic hyperthermia at 43°C (rectal temperature). No pharmacokinetical analyses were performed. Serum analyses of ASAT revealed transient elevation, normalized within a week. All dogs survived 5-FU infusion during normothermia, while 2/8 died after hyperthermia and 1/6 after combined treatment. All deaths occurred within 48 hours after hyperthermia. In the mentioned study the dose of 5-FU must be considered too low to have any impact either on survival or liver toxicity. Furthermore, the temperature in itself (43°C) was high enough to cause all the described changes.

As infusion of 5-fluorouracil is a widely used therapy for colorectal liver metastases it might be tempting to combine it with hyperthermia in the clinical situation. In the present study the high mortality after combined treatment indicates an increased drug toxicity under hyperthermic conditions. Although this study is performed on rats it demonstrates that it is essential to perform further experimental studies in animals before the combination can be given to patients.

Blood flow measurements: In most studies concerning the effect of hyperthermia on experimental tumors the blood flow is proposed to be of great importance. Various methods have been practiced for blood flow measurements, in tumors as well as in normal tissue (Vaupel et al 1980; Dickson and Calderwood 1980; Song 1982). All these studies have in common that only superficial tissues or isolated tumors are studied. The knowledge of blood flow changes in parenchymatous organs during hyperthermia is very limited (Sundqvist et al 1979; Wike-Hooley et al 1981) and more or less contradictory to results obtained in muscle and skin (vide supra).

Liver blood flow measurements have been performed in different experimental and clinical situations using the Xenon clearance technique (Gelin et al 1968; Darle 1970; Sherriff et al 1977). It is considered to be a suitable technique for regional studies. Because of great variations in the intratumorous circulation the interpretation of results obtained after intratumorous injections must be cautious (Pettersson 1979). The technique is rather simple and owes its merit to being repeatable at short intervals in the same subject.

In the present study the differences in the results between analyses carried out with different start-points and with different end-points

of the fit were not in accordance with a pure bi-exponential model. A two-compartment model is a simplification of a more complex wash-out function but for the assessment of relative blood flow in the rat it can be convenient as the discrepancies are small. The wash-out curves obtained could be separated into a fast and a slow component. The liver blood flow was represented by the fast component k_1 while the slow compartment k_2 was supposed to represent blood flow in either heterogeneously perfused liver, or tissue with a larger partition coefficient. k_2 was of the same magnitude for all administration techniques and thus of minor importance for our purpose of detecting relative changes. Large variations in the fast component, k_1 (18-27 %) were found between repeated studies in the same animal. This may be due to physiological variations within the liver or the methodological limitations of the Xenon technique. Variations in the value of k_1 have been observed in the magnitude of 7 % in man (Sherriff et al 1977), 10 % in cat (Hollenberg & Dogerty 1966), 18 % in cat (Darle 1970) and 19 % in rat (Smith and Clarke 1976). This may indicate variations in the hepatic blood flow rather than limitations of the method.

In a study by Pirttiaho et al (1980) ^{133}Xe clearance after i.v. injection was compared with dynamic $^{99\text{m}}\text{Tc}$ -sulphur colloid accumulation and indocyanin green disappearance. The latter methods were found to be comparable, whereas flow measurements with Xenon did not correlate well with the other methods. The administration route of Xenon in this study may be one reason for this result. The difficulty of determining the partition coefficient for liver tissue, necessary for calculation of the actual blood flow was considered to be the most important reason for the diverging results. In the present study the partition coefficient was not included, as only relative changes in blood flow were sought for.

To be able to induce local hyperthermia that was parallel with blood flow measurements the liver lobe had to be exteriorized. This caused a reduction of k_1 of approximately 30 % (V). As this procedure was performed throughout the studies (V, VI) it did not influence the comparison between different measurements.

Blood flow during hyperthermia: Increased blood flow during hyperthermia in superficial tissue has been reported by many investigators, regardless of the technique used (vide supra). Usually blood flow increases at a temperature of 39-41°C with a further increase until it finally decreases above 45°C as a result of heat damage to the vasculature. During total body hyperthermia at 41.5°C the arterial liver blood flow initially decreases and later returns to prehyperthermic level before the end of 60 min heating (Sundqvist et al 1979). The same blood flow pattern has been found in liver tumors. In superficial tumors blood flow has been shown to increase or stay fairly constant at temperatures below 42°C (Vaapel et al 1980; Song 1982) and then to fall considerably at higher temperatures. The different results obtained in rat liver during total body hyperthermia may be explained

by an insufficient penetration of heat or by special regulatory mechanisms in the liver tissue.

During local hyperthermia (42°C) in the exteriorized liver lobe (VI) a decrease in blood flow was found. As the temperature in this study was well controlled this indicates a fundamental difference in the vascular response to heat between the liver and superficial tissue. In normal liver parenchyma blood flow returned to prehyperthermic levels within 30 min after hyperthermia. A remaining decrease in tumor blood flow may indicate at least a temporary disturbance in tumor circulation, caused by local hyperthermia.

Changes in liver blood flow during hyperthermia may explain the sensitivity of liver tissue to heat, as observed in in vivo experimental situations (Fletcher et al 1982) as well as in clinical observations (Hicks et al 1948). Total body hyperthermia as well as hyperthermic perfusion of the entire liver may endanger the functional properties of the organ. With induction of hyperthermia to a restricted part of the liver - local hyperthermia - this danger can be avoided. Sufficient differential heating may be achieved in liver tumors because of a blood flow that is lower than that in normal parenchyma. A tumoricidal rise of intratumoral temperature can then be obtained in different parts of the liver at different sessions of the treatment with the goal of achieving tumor reduction even when scattered metastases are at hand.

CONCLUSIONS

The present study introduces a technique for induction of general and local hyperthermia in small experimental animals by microwave irradiation. The problem of microwaves disturbing the continuous control of temperature levels is solved by alternating irradiation with temperature registrations. In that way exact heating is achieved. A microprocessor based feed-back system facilitates the hyperthermia treatment.

With total body hyperthermia in rats a maximum temperature level of 41.5°C was essential, irrespective of the number of exposures. At higher temperatures evidence of liver cell damage and increased lysosomal activity occurred, indicating a sensitivity of the liver to higher temperatures. No tumoricidal effect of total body hyperthermia could be demonstrated when studied in inoculated liver tumors.

In local hyperthermia treatment of liver tumors a tumoricidal effect was demonstrated, confirmed by tumor volume measurements and histopathological examinations. With increasing tumor volume an increase in the development of tumor necrosis was found. The tumoricidal effect on liver tumors may be a result of selective heating of tumor tissue caused by decreased dissipation of heat.

Local hyperthermia of the liver affected the response to i.v. infusion of 5-fluorouracil. An increased toxicity and a faster decline in the serum concentration curve of 5-FU indicated that drug metabolism was affected by heat. No increase in the tumoricidal effect could be demonstrated after combination of hyperthermia and 5-FU infusion compared with hyperthermia alone.

With the purpose of investigating blood flow changes in the liver during hyperthermia, the 133-Xenon clearance method was adopted for studies in rats. The reliability of the method was tested by comparing different ways of isotope administration. Great variations in individual blood flow measurements were found. Nevertheless, the simplicity and the possibility of repeated measurements made the method valuable for the present purpose.

During local hyperthermia treatment liver blood flow studies were performed by the above-mentioned method. An overall decrease in blood flow was demonstrated during hyperthermia. In liver tumors the blood flow did not return to prehyperthermic values after heating, suggesting at least a temporary disturbance in tumor circulation.

FUTURE ASPECTS

In total body hyperthermia the difference in temperature between the tumor and normal tissue is probably small. This makes the technique less suitable for the treatment of liver tumors. In order to reach a sufficient temperature in the liver tumor the body temperature must be kept at a high level. Thereby the liver parenchymal function as well as other vital functions may be endangered.

Regional hyperthermic perfusion of the liver will induce a high enough temperature in the liver, but may at the same time cause thermal damage to the normal parenchyma. Furthermore, this technique is accomplished by advanced surgical procedures.

With the induction of hyperthermia to a restricted part of the liver - local hyperthermia - an unfavorable impact on the liver function can be avoided. At present no reliable technique is available for the administration of local hyperthermia to the human liver under controlled conditions. In future experimental and clinical work efforts must be made to solve the technical problems of selective heating. One possible alternative may be implantation of intraabdominal heat producing devices at laparotomy. More practical would be external heat sources with the capacity to produce exact induction of heat into small volumes of tissue, maybe combined with some kind of sensitization of tumor tissue or by labelling the tumor tissue with metallic particles acting as "hot spots".

As the blood flow plays a central role in the action of the hyperthermia, manipulations with the liver circulation, like temporary dearterialization and blockage of arterial nutrition by microspheres, must be studied in connection with local hyperthermia.

Drugs that gain an enhanced chemotherapeutic effect with increasing temperatures may add a further dimension to the possible beneficial effects of local hyperthermia.

ACKNOWLEDGEMENTS

The studies were supported by grants from the Swedish Cancer Society (grants no 98-B80-15XA and 513-B83-10XB), John and Augusta Persson Foundation for Cancer Research, Segerfalks Foundation, the Medical Faculty of the University of Lund.

REFERENCES

- Allan BD, Norman RL. Hyperthermia and cancerous tissue water structure.
Cancer Chemother Rep 58:296-298, 1974.
- Allen FM. Biological modification of effects of roentgen rays.
Am J Roentgenol Rad Ther Nucl Med (NS) 73:836-848, 1955.
- Almersjö O, Bengmark S, Hafström Lo. Liver resection for cancer.
Acta Chir Scand 142:139-144, 1976.
- Andersen AM, Ladefoged J. Partition coefficient of Xe-133 between various tissues and blood in vivo.
J Clin Lab Invest 19:72-78, 1967.
- Arcangeli G, Barni E, Cividalli A, Mauro F, Morelli D, Nervi C, Spano M, Tabocchini A. Effectiveness of microwave hyperthermia combined with ionizing radiation. Clinical results on neck node metastases.
J Radiat Oncol Biol Phys 6:143-148, 1980.
- Auda SP, Steinert HR, Elias EG, Viravathana T. Selective tumor heating by short wave radiofrequency (rf).
Cancer 46:1962-1968, 1980.
- Baker RJ, Smith V, Phillips TL, Kane LJ, Cope LH. A system for developing microwave-induced hyperthermia in small animals.
Trans Microwave Theory Tech MTT-26, 541-545, 1978.
- Ballard BE. Pharmacokinetics and temperature.
J Pharm Sci 63:1345-1358, 1974.
- Bengmark S, Börjesson B, Hafström Lo. The natural history of primary carcinoma of the liver.
Scand J Gastroenterol 6:351-355, 1971.
- Bengmark S, Hafström Lo. The natural history of primary and secondary malignant tumors of the liver.
Cancer 23:198-202, 1969.
- Bengtsson G, Carlsson G, Hafström Lo, Jönsson P-E. Natural history of patients with untreated liver metastases from colorectal cancer.
Am J Surg 141:586-589, 1981.
- Birnie JH, Grayson J. Observations on temperature distribution and liver blood flow in the rat.
J Physiol 116:189-201, 1952.

Boddie AW, Booker L, Mullins JD, Buckley CJ, McBride CM. Hepatic hyperthermia by total isolation and regional perfusion in vivo. J Surg Res 26:447-457, 1979.

Bowers W, Hubbard R, Wagner D, Chisholm P, Murphy M, Leav I, Hamlet M, Maher J. Integrity on perfused rat liver at different heat loads. Lab Invest 44:99-104, 1981.

Brauer RW, Balam RW, Bond HE, Carroll HW, Griesham JW, Pessotti RL. Reversible and irreversible changes in liver at temperatures approaching critical upper levels. Fed Proc 22:724-728, 1963.

Bull JM, Lees D, Schuette W, Whang-Peng J, Smith R, Binum G, Atkinson R, Gottdiener JS, Gralnick HR, Shawker TH, DeVita V. Whole body hyperthermia: a phase-I trial of a potential adjuvant to chemotherapy. Ann Int Med 90:317-323, 1979.

Busch W. Über den Einfluss, welchen heftigere Erysipeln zuweilen auf organisierte Neubildungen aus üben. Verhandl Naturh Preuss Rhein Westphal 23:28-30, 1866.

Cancer Incidence in Sweden 1979.

The Cancer Registry, National Board of Health and Welfare, Sweden. Published 1982.

Carlsson G. Hepatic tumor growth. Influence of hepatic artery occlusion. An experimental study in rats. Bulletin no 30 From the Department of Surgery, University of Lund, Sweden, 1982.

Carlsson G, Ekelund L, Stigsson L, Hafström Lo. Vascularization and tumor volume estimations of single liver tumors in rats. Ann Chir Gynecol Fenn, in press 1983.

Carlsson G, Hafström Lo, Hugander A, Jönsson P-E, Bolmsjö M, Persson B. The effect of total body microwave hyperthermia and hepatic artery ligation on liver tumors - an experimental study in rats. J Surg Oncol 22:37-40, 1983.

Cater EB, Silver IA, Watkinson DA. Combined therapy with 220 kv roentgen and 10 cm microwave heating in rat hepatoma. Acta Radiol Ther Phys Biol (NS) 2:321-336, 1964.

Cavaliere R, Ciocatto EC, Giovanella BC, Heidelberger C, Johnson RO, Margottini M, Mondovi B, Moricca G, Rossi-Fanelli A. Selective heat sensitivity of cancer cells. Biochemical and clinical studies. Cancer 20:1351-1381, 1967.

- Coley WB. The treatment of malignant tumors by repeated inoculations of erysipelas: with a report of 10 original cases.
Am J Med Sci 105:487-511, 1893.
- Crandall ED, Critz AM, Osher AS, et al. Influence of pH on elastic deformability of the human erythrocyte membrane.
Am J Physiol 235:C269-C278, 1978.
- Crile G Jr. Heat as an adjunct to the treatment of cancer: experimental studies.
Cleveland Clin Quart 28:75, 1961.
- Crile G Jr. Selective destruction of cancers after exposure to heat.
Ann Surg 156:404-407, 1962.
- Daly JM, Smith G, Frazier OH, Dudrick SJ, Copeland EM. Effects of systemic hyperthermia and intrahepatic infusion with 5-fluorouracil.
Cancer 49:1112-1115, 1982.
- Darle N. Xenon-133 clearance and liver blood flow. An experimental study in the cat.
Acta Chir Scand suppl 407, 1-64, 1970.
- Dickson JA, Calderwood SK. Temperature range and selective sensitivity of tumors to hyperthermia: a critical review.
Ann NY Acad Sci 335:180-205, 1980.
- Dickson JA, Shah DM. The effect of hyperthermia (42°C) of the biochemistry and growth of a malignant cell line.
Eur J Cancer 8:561-571, 1972.
- Dickson JA, Shah SA. Technology for the hyperthermic treatment of large solid tumors at 50°C.
Clin Oncol 3:301-318, 1977.
- Dickson JA, Shah SA. Hyperthermia and the immune response in cancer therapy.
Cancer Immunol Immunother 9:1-10, 1980.
- Dickson JA, Suzangar M. In vitro- in vivo studies on the susceptibility of the solid yoshida sarcoma to drugs and hyperthermia.
Cancer Res 34:1263-1274, 1974.
- Dietzel F, Seibert G, Globe G. Der Einfluss einer Hochfrequenz-Hyperthermie auf den Verlauf den Erlich-aszites-Karzinoms der Maus.
Strahlentherapie 149:105-117, 1975.
- Draper N, Smith H. In: Applied regression analyses, John Wiley Sons Inc., New York. pp 267-270, 1966.

Field SB, Bleehe NM. Hyperthermia in the treatment of cancer.
Cancer Treatm Rev 6:63-94, 1979.

Fisher B, Fisher ER. Experimental studies on factors influencing
hepatic metastases.
Ann Surg 150(2):731-744, 1959.

Fletcher AM, Chetas TC, Dewhurst MW, Wilson SE. Liver temperatures in
rats subjected to whole body hyperthermia: evidence for congestion and
lymphocyte damage.
Natl Ca Inst Monogr 61:231-233, 1982.

Gelin L-E, Lewis DH, Nilsson L. Liver blood flow in man during
abdominal surgery. Description of a method utilizing intrahepatic
injections of radioactive Xenon. Normal values and effect of temporary
occlusion.
Acta Hepatosplenol 15:13-24, 1968.

Gessler AE, McCarty KS, Parkinson MC. Erradication of spontaneous
mouse tumors by high frequency of radiation.
Exp Med Surg 8:143-167, 1950.

Giles U. The historic development and modern application of artificial
fever.
New Orleans Med Soc J 91:655-670, 1938.

Giovanella BC, Stehlin Jr JS, Morgan AC. Selective lethal effect of
supranormal temperatures on human neoplastic cells.
Cancer Res 36:3944-3950, 1976.

Gullino PM. Influence of blood supply on thermal prosperities and
metabolism of mammary carcinomas.
Ann NY Acad Sci 335:1-21, 1980.

Gustafsson B, Almersjö O, Baldersten A, Hasselgren, P-O. New assay of
5-fluorouracil in serum by isotachophoresis.
J Chromatogr 179:151-159, 1979.

Hafström Lo, Ehrson H, Hugander A, Jönsson P-E, Westling H. Regional
hyperthermic perfusion for malignant melanoma.
In: Progress in Clinical and Biological Research (ed M Gauthiere, E
Albert), vol 107, Biomedical Thermology, Alan R. Liss Inc, NY, pp
793-798, 1982.

Hafström Lo, Jönsson P-E, Bolmsjö M, Persson B. Tolerance of induced
hyperthermia. Experiments in rats with liver tumors subjected to
fractionated exposures of microwaves.
In: Proc 1st Meeting of European Group of Hyperthermia in Radiation
Oncology (Arcangeli G, Mauro F, ed), Cambridge 193-197, 1979.

- Hall RR, Schade ROK, Swinney J. Effects of hyperthermia on blood cancer.
Brit Med J 2:593-594, 1974.
- Hicks MH, Holt HP, Guerrant JL, Leavell BS. The effect of spontaneous and artificially induced fever on liver function.
J Clin Invest 27:580-587, 1948.
- Hollenberg M, Dougherty J. Liver blood flow measured by portal venous and hepatic arterial routes with Kr-85.
Am J Physiol 210:926-932, 1966.
- Honess DJ, Bleeheh NM. Therapeutic ratios for the combination of whole-body hyperthermia with chemotherapy in mice (Abs).
Strahlentherapie 159:374, 1983.
- Hornback NB, Shupe RE, Shidnia H, Joe BT, Sayoc E, Marshall C. Preliminary clinical results of combined 433 MHz microwave therapy and radiation therapy on patients with advanced cancer.
Cancer 40:2854-2863, 1977.
- Hultberg B, Isaksson A, Jansson L. β -hexosaminidase in serum from patients with cirrhosis and cholestasis.
Enzyme 26:296-300, 1981.
- Johnson HJ. The action of short radiowaves on tissues: III A comparison of the thermal sensitivities of transplantable tumors in vivo and in vitro.
Am J Cancer 38:533, 1940.
- Kang MS, Rhee JG, Levitt SH, Song CW. Cytotoxic effect of hyperthermia on tumor cells in vivo.
Natl Ca Inst Monogr 61:157-159, 1982.
- Kim JH, Hahn EW, Tokita M. Combination hyperthermia and radiation therapy for cutaneous malignant melanoma.
Cancer 41:2143-2148, 1978.
- Kummer D, Kraml F. Studies of the thymidine-triphosphate synthesis in malignant tumors. II. Effect of hyperthermia, vitamin K and cytotoxic agents.
Zeitschr Krebsforsch Klin Onk 88:145-156, 1977.
- Krementz ET, Carter RD, Sutherland CM, Hutton I. Chemotherapy of sarcomas and limbs by regional perfusion.
Ann Surg 185:555-564, 1977.
- Larkin JM, Edwards WS, Smith DE, Clarke PJ. Systemic thermotherapy: description of a method of physiologic tolerance in clinical subjects.
Cancer 40:3155-3159, 1977.

- Lehmann JF. "Diathermy" in Handbook of Physical Medicine and Rehabilitation pp 273-345, 1971.
- Leveen HH, Wapnick S, Piccone VA, Falk G, Ahmed N. Tumor eradication by radiofrequency therapy.
JAMA 235:2198-2200, 1976.
- Lin PS, Wallach FH, Tsai S. Temperature-induced variations in the surface topology of cultured lymphocytes are revealed by scanning electron microscopy.
Proc Natl Acad Sci US 70:2492-2496, 1973.
- Lindholm CE, Kjellén E, Landberg T, Nilsson P, Persson B. Microwave-induced hyperthermia and ionizing radiation.
Acta Radiol Onc 21:241-254, 1982.
- Loshek DP. Pulsed microwave thermometry: an algorithm for thermal perturbation.
Natl Ca Inst Monogr 61:517-519, 1982.
- Magin RL, Sysyk RL, Litterst CL. Distribution of adriamycin in mice under conditions of local hyperthermia which improve systemic drug therapy.
Cancer Treatm Rep 64:203-210, 1980.
- Marmor JB. Interactions of hyperthermia and chemotherapy in animals.
Cancer Res 39:2269-2276, 1979.
- Marmor JB, Kozak D, Hahn GM. Effects of systemically administered bleomycin or adriamycin with local hyperthermia on primary tumor and lung metastases.
Cancer Treatm Rep 63:1279-1290, 1979.
- Mathie RT. Measurement of liver blood flow in man.
In: Blood Flow Measurement in Man, Castle House Publications pp 139-149, 1982.
- Mendecki J, Friedenthal E, Botstein C. Effects of microwave induced local hyperthermia on mammary adenocarcinoma in C3H mice.
Cancer Res 36:2113-2114, 1976.
- Mimnaugh EG, Waring RW, Sikic BI, Magin RL, Drew R, Litterst CL, Gram TE, Guarino AM. Effect of whole body hyperthermia on the disposition and metabolism of adriamycin in rabbits.
Cancer Res 38:1420-1425, 1978.
- Mizuno S, Amagai M, Ishida A. Synergistic cell killing by antitumor agents and hyperthermia in cultured cells.
Gann 71:471-478, 1980.

Mondovi B, Strom R, Rotilio G, Finazzi Agro' A, Cavaliere R, Rossi-Fanelli A. The biochemical mechanism of selective heat sensitivity of cancer cells. I. Studies on cellular respiration. Eur J Cancer 5:129-136, 1969. II. Studies on nucleic acids and protein synthesis. Eur J Cancer 5:137-146, 1969.

Muckle DF, Dickson JA. Hyperthermia (42°C) as an adjuvant to radiotherapy and chemotherapy in the treatment of the allogeneic VX2 carcinoma in the rabbit. Brit J Cancer 27:307-315, 1973.

Ostrow S, van Echo D, Whitacre M, Aisner J, Simon R, Wienik PH. Physiologic response and toxicity in patients undergoing whole body hyperthermia for the treatment of cancer. Cancer Treatm Rep 65:323-325, 1981.

Overgaard J. Ultrastructure of a murine mammary carcinoma exposed to hyperthermia in vivo. Cancer Res 36:983-995, 1976.

Overgaard J. Combined adriamycin and hyperthermia treatment of a murine mammary carcinoma in vivo. Cancer Res 36:3077-3081, 1976.

Overgaard J. Effects of hyperthermia on malignant cells in vivo. Cancer 39:2637-2646, 1977.

Overgaard J, Bichel P. The influence of hypoxia and acidity on the hyperthermic response on malignant cells in vitro. Radiology 123:511-514, 1977.

Overgaard K, Okkels H. The action of heat on Wood's sarcomas. Acta Radiol 21:577-582, 1940.

Overgaard K, Overgaard J. Investigations on the possibility of a thermic tumor therapy. I. Short wave treatment of a transplanted isologous mouse mammary carcinoma. Eur J Cancer 8:65-78, 1972. Investigations on the possibility of a thermic tumor therapy. II Action of combined heat-roentgen treatment on a transplanted mouse mammary carcinoma. Eur J Cancer 8:573-575, 1972.

Palzer RJ, Heidelberger C. Studies on the quantitative biology of hyperthermic killing of HeLa cells. Cancer Res 33:415-421, 1973.

Parks LC, Minaberry D, Nely B et al. Extracorporeal-induced systemic hyperthermia: effects on man and cancer. Surg Forum 29:148-150, 1978.

- Petersson H-I. Tumor blood flow compared with normal tissue blood flow. In: Tumor Blood Circulation, pp 103-114, CRC-Press Inc., 1979.
- Pettigrew RT, Galt JM, Ludgate CM, Smith AN. Clinical effects of whole body hypothermia in advanced malignancy. Brit Med J 4:679-682, 1974.
- Pirttiaho H, Pitkänen U, Rajasalmi M, Ahonen A. Comparison of three methods of measuring liver blood flow. Acta Radiol Diagn 21:535-539, 1980.
- Roti-Roti JL. Heat induced cell death and radiosensitization: molecular mechanisms. Natl Ca Inst Monogr 61:3-10, 1982.
- Scand Society for Clinical Chemistry and Clinical Physiology: The Committee of Enzymes of the .. Scand J Clin Lab Invest 33:291, 1974.
- Sherriff SB, Smart RC, Taylor I. Clinical study of liver blood flow in man measured by ¹³³Xenon clearance after portal vein injection. Gut 18:1021-1031, 1977.
- Shibata HR, MacLean LD. Blood flow to tumors. Prog Clin Cancer 2:33-47, 1966.
- Shingleton WW. Selective heating and cooling of tissue in cancer chemotherapy. Ann Surg 156:408-414, 1962.
- Short JG, Turner PF. Physical hyperthermia and cancer therapy. Proc IEE 68:103-142, 1980.
- Sieck R, Roth B, Vahs N. Kinetics of rat RES by intravenously injected zymosan. In: Kupffer Cells and Other Liver Sinusoidal Cells (Wisse E and Knook DL, eds), Elsevier North-Holland Biomedical Press, Amsterdam 1977.
- Skibba JL, Collins FG. Effect of temperature of biochemical functions in the isolated perfused rat liver. J Surg Res 24:435-441, 1978.
- Slotman GJ, Milazzo J, Jain K, Swaminathan AP, Rush BF. The effect of radiofrequency hyperthermia on the CA 755 murine adenocarcinoma. Cancer 46:1992-1995, 1980.
- Smith A, Clarke MB. The determination of hepatic blood flow in the rat using Xenon-133. Int J Appl Rad Isot 27:201-210, 1976.

Song CW. Physiological factors in hyperthermia.
Natl Ca Inst Monogr 61:169-176, 1982.

Steele Jr G, Sjögren HO. Cross-reacting tumor associated antigen(s) among chemically induced rat colon carcinomas.
Ca Res 34:1801-1807, 1974.

Stehlin Jr JS, Giovannella BD, de Ipolye P, Muenez LR, Anderson RF. Results of hyperthermic perfusion for melanoma of the extremities.
Surg Gynecol Obstet 140:338, 1975.

Storm FK, Harrison WH, Elliott RS, Morton DL. Normal tissue and solid tumor effects of hyperthermia in animal models and clinical trials.
Cancer Res 39:2245-2251, 1979.

Storm FK, Kaiser LR, Goodnight JE, Harrison WH, Elliott RS, Gomes AS, Morton DL. Thermochemotherapy for melanoma metastases in the liver.
Cancer 49:1243-1248, 1982.

Sundqvist K. Blood flow in experimental tumors. With special reference to the effect of vasoactive drugs. A study in the rat.
Bulletin no 21 from the Department of Surgery, University of Lund, Sweden, 1980.

Sundqvist K, Bolmsjö M, Hafström L, Jönsson P-E, Nilsson P, Persson B. Influence of microwave hyperthermia on blood flow in experimental liver tumors.
In: Proceedings of the 1st Meeting of European Group of Hyperthermia in Radiation Oncology (Arcangeli G, Mauro F, eds), Cambridge 1979, pp 215-219.

Sundqvist K, Hafström L, Persson B. Measurement of total and regional tumor blood flow and organ blood flow using ^{99m}Tc -labelled microspheres.
Eur Surg Res 10:433-443, 1978.

Suzuki K. Application of heat to cancer chemotherapy - experimental studies.
Nagoja J Med Sci 30:1-21, 1967.

Taylor I. A critical review of the treatment of colorectal liver metastases.
Clin Oncol 8:149-158, 1982.

Turano C, Ferraro A, Strom R, Cavaliere R, Rossi-Fanelli A. The biochemical mechanism of selective heat sensitivity of cancer cells. III Studies on lysosomes.
Eur J Cancer 6:67-72, 1970.

Warren SL. Preliminary studies of the effect of artificial fever upon hopeless tumor cases.

Am J Roentgenol 33:75-87, 1935.

Vaupel P, Thews G. Pathophysiological aspects of glucose uptake by the tumor tissue under various conditions of oxygen and glucose supply. Adv Exp Med Biol 75:547-553, 1976.

Vaupel P, Ostheimer K, Muller-Klieser W. Circulatory and metabolic response of malignant tumors during localized hyperthermia. J Cancer Res Clin Oncol 8:15-29, 1980.

Westermark F. Über die Behandlung des Ulcerierenden Cervixkarzinoms mittels konstanter Wärme. Zblt Gyn 49:1335-1339, 1898.

Westermark N. The effect of heat upon rat tumors. Scand Arch Physiol 52:257-322, 1927.

Westra A, Dewey WC. Variation on sensitivity to heat shock during cell cycle of Chinese hamster cells in vitro. Int J Radiat Biol 119(5):467-477, 1971

Wike-Hooley JL, Faithfull NS, Pekelharing JM, van den Berg AP. Whole body hyperthermia and hepatic toxicity. Strahlentherapie 157:623(abstr), 1981.

Wills EJ, Findlay JM, McManus JPA. Effects of hyperthermia therapy on the liver. II Morphological observations. J Clin Pathol 29:1-10, 1976.

Woeber K. The effect of ultrasound in the treatment of cancer. In: The Ultrasonic Energy (ed Kelly, Illinois University), Illinois Press, pp 138-149, 1965.

von Ardenne M. Selective multiphase cancer therapy: conceptual aspects and experimental basis. Adv Pharmacol Chemother 10:339-380, 1972.

von Ardenne M, Reitnauer PG. Selective occlusion of cancer tissue capillaries as the central mechanism of the cancer multistep therapy. Jpn J Clin Onc 10:31-48, 1980.

Zander R, Schmid-Schönbein H. Influence of intracellular convention on the oxygen release by human erythrocytes. Pflügers Arch 335:58-73, 1972.

PRINTING
ILLUSTRATION OF DEPARTMENT
DEPARTMENT OF SURGERY
UNIVERSITY OF LUND
1983.